2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of diazinon. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure-inhalation, oral, and dermal-and then by health effect-death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic. These data are discussed in terms of three exposure periods-acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowestobserved-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into “less serious” or “serious” effects. “Serious” effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). “Less serious” effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, “less serious” LOAEL, or “serious” LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt

at distinguishing between “less serious” and “serious” effects. The distinction between “less serious” effects and “serious” effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the LSE (Levels of Significant Exposure) tables and figures may differ depending on the user’s perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for diazinon. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute-, intermediate-, and chronic-duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User’s Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

2.2.1 Inhalation Exposure

Diazinon has a low volatility, thus inhalation exposure is likely to be to diazinon aerosols rather than vapor. In one of the studies described below, animals were exposed to diazinon in inhalation chambers (Holbert 1989). It is possible that some of the exposure under these conditions was by the dermal route and/or the oral route (grooming).

2.2.1.1 Death

There are no reports of deaths in humans or animals exposed by inhalation to diazinon alone. But one clinical study reported human death following inhalation exposure to an insecticide mixture that contained diazinon and malathion, another anticholinesterase insecticide. A 5 l-year-old man died from cardiac arrest, despite atropine therapy, following inhalation exposure to a commercial insecticide formulation containing diazinon and malathion. Autopsy revealed mild pathologic changes in intercostal muscle tissue, including muscle fibers with subsarcolemmal grouped granular basophilic inclusions and scattered areas of necrosis. The victim's neuromuscular acetylcholinesterase activity was one-half that of muscle from unexposed persons (Wecker et al. 1985).

No deaths were reported in Sprague-Dawley rats (5 of each sex) exposed to 2,330 mg/m^3 diazinon for 4 hours in inhalation chambers and observed for a further 14 days (Holbert 1989), or in hybrid rats (groups of 10 of each sex) exposed to air concentrations of 0.05, 0.46, 1.57, or 11.6 mg/m^3 diazinon (nose-only) for 6 hours a day, 5 days a week for 3 weeks (Hartman 1990).

2.2.1.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, endocrine, dermal, ocular, or body weight effects in humans after inhalation exposure to diazinon. A single study described mild degenerative changes in the muscles in a human acute-duration exposure to a mixture of diazinon and malathion (Wecker 1985). No studies were located regarding gastrointestinal, musculoskeletal, dermal, or metabolic effects in animals after inhalation exposure to diazinon. The systemic effects observed in humans and animals after inhalation exposure to diazinon are discussed below. The highest NOAEL and all LOAEL values from each reliable study

for systemic end points in each species and duration category are recorded in Table 2-l and plotted in Figure 2- 1.

Respiratory Effects. Nasal discharge was observed in Sprague-Dawley rats exposed to 2,330 mg/m^3 diazinon for 4 hours in an inhalation chamber (Holbert 1989). A statistically significant increase in lung to body weight ratio was observed in hybrid female rats exposed to 0.46 and 1.57 mg/m^3 diazinon (nose-only) for 3 weeks, 5 days a week for 6 hours a day (Hartman 1990). This effect was not seen in male rats or in female rats exposed at 11.6 mg/m^3, so its toxicological significance is unclear. No gross or histological evidence of treatment-related damage to nasal tissues or the lungs was observed at the termination of this study at any concentration of diazinon.

Cardiovascular Effects. No gross or histological evidence of treatment-related damage to the heart was observed in hybrid rats (10 of each sex) exposed to 11.6 mg/m^3 diazmon (nose-only) for 3 weeks, 5 days a week for 6 hours a day (Hartman 1990).

Hematological Effects. No statistically significant differences in hematological parameters (erythrocyte count, hemoglobin, packed red cell volume) were seen compared to controls in hybrid rats (10 of each sex) exposed to up to 11.6 mg/m^3 diazinon (nose-only) for 3 weeks, 5 days a week for 6 hours a day (Hartman 1990).

Musculoskeletal Effects. Mild pathologic changes in the intercostal muscle tissue, including muscle fibers with subsarcolemmal grouped granular basophilic inclusions and scattered areas of necrosis were reported in the autopsy of a 51-year-old man who died from high acute-duration exposure, via inhalation, to a commercial insecticide spray containing diazinon and malathion. Neuromuscular acetylcholinesterase activity was one-half that of muscle from unexposed persons (Wecker et al. 1985).

Hepatic Effects. - No gross or histological evidence of treatment-related damage to the liver was observed in hybrid rats (10 of each sex) exposed to 11.6 mg/m^3 diazinon (nose-only) for 3 weeks, 5 days a week for 6 hours a day (Hartman 1990).

Renal Effects. Polyuria was observed in Sprague-Dawley rats exposed to 2,330 mg/m^3 for 4 hours (Holbert 1989). No gross or histological evidence of treatment-related damage to the kidney was

Table 2-1. Levels of Significant Exposure to Diazinon - Inhalation

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
ACUTE EXPOSURE							
Systemic							
1	Rat (Sprague-Dawley)	4 hr	Resp		2330 M (nasal discharge; 3/5)		Holbert 1989
			Renal		2330 F (polyuria; 3/5)		
			Bd Wt	2330			
Neurological							
2	Rat (Sprague-Dawley)	4 hr			2330 (decreased activity, 2/5; salivation, 2/5)		Holbert 1989
INTERMEDIATE EXPOSURE							
Systemic							
3	Rat (Hybrid)	3 wk 5 d/wk 6 hr/d	Resp	11.6			Hartman 1990
			Cardio	11.6			
			Hemato	11.6			
			Hepatic	11.6			
			Renal	11.6			
			Endocr	11.6			
			Ocular	11.6			
			Bd Wt	11.6			
Immunological/Lymphoreticular							
4	Rat (Hybrid)	3 wk 5 d/wk 6 hr/d		11.6			Hartman 1990

Table 2-1. Levels of Significant Exposure to Diazinon - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
	Neurological						
5	Rat (Hybrid)	3 wk 5 d/wk 6 hr/d		0.46[b] F	1.57 F (20% decrease in brain AChE)		Hartman 1990

[a]The number corresponds to entries in Figure 2-1.

[b]Used to derive an intermediate-duration Minimal Risk Level (MRL) of 0.009 mg/m^3; based on the NOAEL of 0.46 mg/m^3 for brain acetylcholinesterase inhibition; concentration adjusted for intermittent exposure, converted to a human equivalent concentration, and divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability).

AChE = acetylcholinesterase; Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; gen = generation; Hemato = hematological; hr = hour(s); LOAEL = lowest-observable-adverse-effect level; NOAEL = no-observable-adverse-effect level; Resp = respiratory; wk = week(s)

Figure 2-1. Levels of Significant Exposure to Diazinon - Inhalation

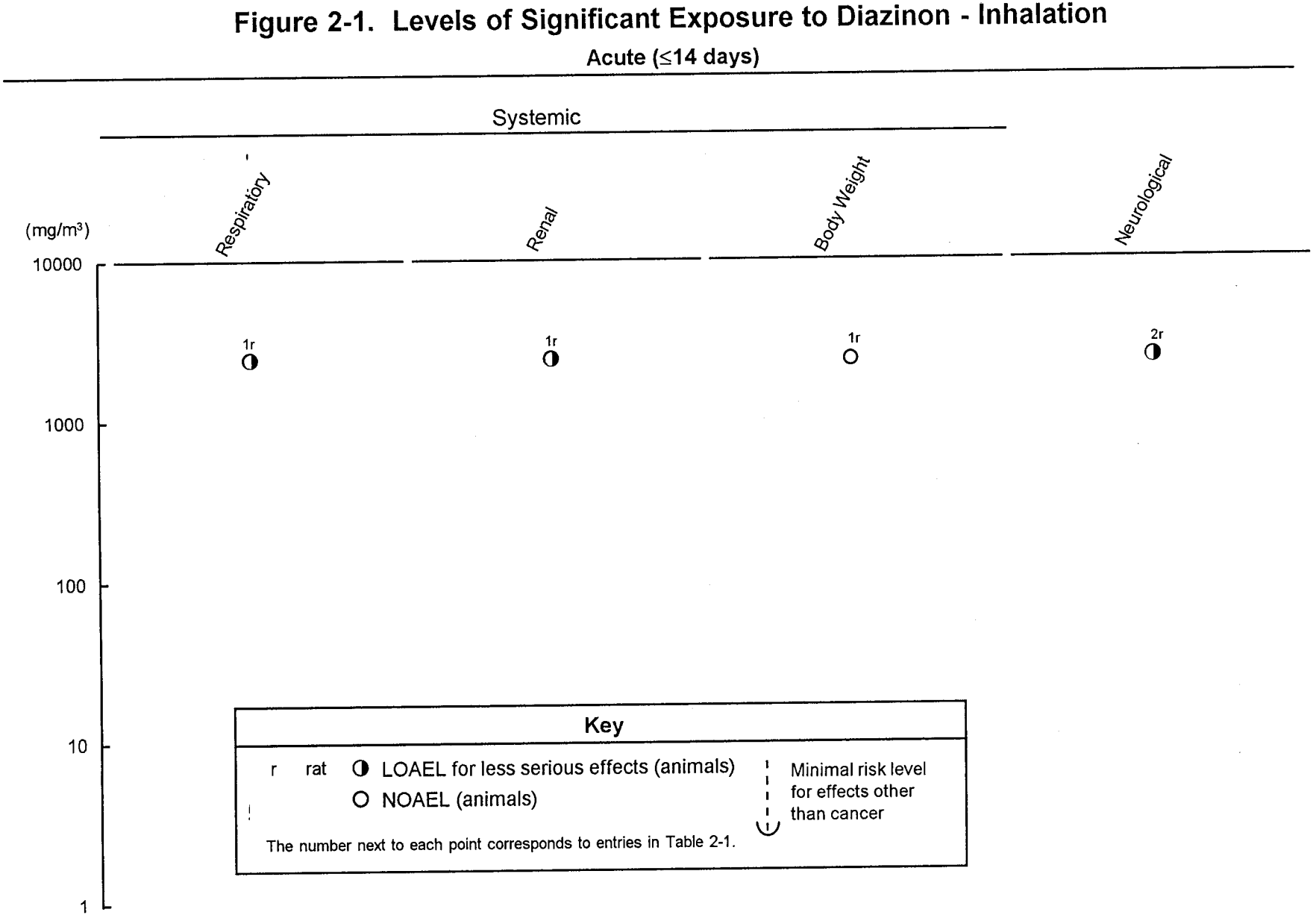
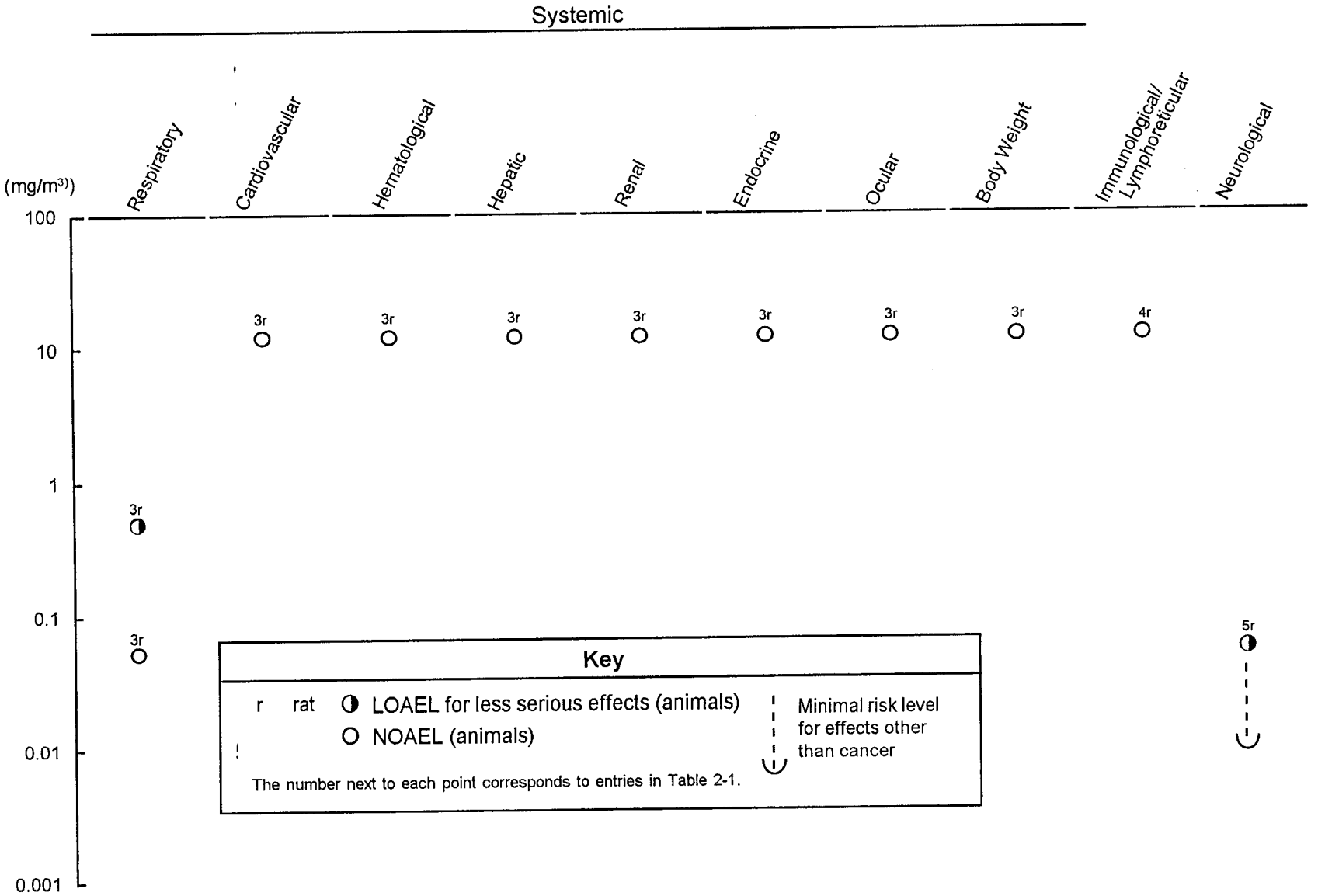


Figure 2-1. Levels of Significant Exposure to Diazinon - Inhalation (cont.)

Intermediate (15-364 days)



observed in hybrid rats (10 of each sex) exposed to 11.6 mg/m^3 diazinon (nose-only) for 3 weeks, 5 days a week for 6 hours a day (Hartman 1990).

Endocrine Effects. No gross or histological evidence of treatment-related damage to the adrenal gland was observed in hybrid rats (10 of each sex) exposed to 11.6 mg/m^3 diazinon (nose-only) for 3 weeks, 5 days a week for 6 hours a day (Hartman 1990).

Ocular Effects. Ptosis was observed in Sprague-Dawley rats exposed to 2,330 mg/m^3 for 4 hours in an inhalation chamber (Holbert 1989). No evidence of treatment-related ophthalmoscopic lesions was observed in hybrid rats (10 of each sex) exposed to up to 11.6 mg/m^3 diazinon (nose-only) for 3 weeks, 5 days a week for 6 hours a day (Hartman 1990).

Body Weight Effects. No effect on body weight was observed in Sprague-Dawley rats (5 of each sex) exposed to 2,330 mg/m^3 for 4 hours and observed for a further 14 days (Holbert 1989) or in hybrid rats (10 of each sex) exposed to up to 11.6 mg/m^3 diazinon (nose-only) for 3 weeks, 5 days a week for 6 hours a day (Hartman 1990).

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans after inhalation exposure to diazinon.

No gross or histological evidence of treatment-related damage to the spleen was observed in hybrid rats (10 of each sex) exposed to 11.6 mg/m^3 diazinon (nose-only) for 3 weeks, 5 days a week for 6 hours a day (Hartman 1990).

The NOAEL for immunological and/or lymphoreticular end points in hybrid rats for intermediateduration exposure is. recorded in Table 2- 1 and plotted in Figure 2- 1.

2.2.1.4 Neurological Effects

Diazinon, an anticholinesterase organophosphate, inhibits acetylcholinesterase in the central and peripheral nervous system. Inhibition of acetylcholinesterase results in accumulation of acetylcholine

at muscarinic and nicotinic receptors leading to peripheral and central nervous system effects. These effects usually appear within a few minutes to 24 hours after exposure, depending on the extent of exposure. Most of the located reports of incidents of human exposure to diazinon involved occupational exposure via the inhalation route, although it is possible that significant exposure also took place via the dermal route.

Cholinergic symptoms began within 15 minutes in all but one of 18 mushroom workers exposed to diazinon sprayed around the only entrance to a room in which they were working. The workers exhibited reduced serum and erythrocyte cholinesterase levels (markers for diazinon exposure) within 48 hours; serum cholinesterase levels were inhibited 27-29% by diazinon exposure within 15 days post-exposure (Coye et al. 1987). In another report, members of a family complained of signs and symptoms of insecticide poisoning (headache, vomiting, fatigue, chest heaviness) after moving into a house that had been treated with diazinon. Five months after the house had been treated with diazinon, analysis of the family members' urine samples showed "very high urinary levels" (0.5-1.5 mg/L) of a diazinon metabolite, diethyl phosphate (DEP), while serum cholinesterase levels were slightly depressed (79-94% of normal levels). Surface concentrations in the home ranged from 126 to 1,051 μg/m^2, air concentrations were between 5 and 27 ug/m^3, and some clothing showed contamination (0.5-0.7 μg/g). After clean-up of the house, the signs and symptoms reported by family members promptly ceased, and the urinary excretion of DEP dropped to background levels (Richter et al. 1992). Another case study of 99 individuals who were occupationally exposed to diazinon granules 8 hours per day for 39 days during an insecticide application program reported only slight neurological functional deficits (post-shift symbol-digit speed and pattern memory accuracy) as a result of the exposure. A dose of 0.02 mg/kg/day, considered a NOAEL, was estimated for the workers on the basis of measured diazinon concentration in passive dermal badges, hand rinses, and full-shift breathing-zone air samples. Thus, multiple exposure routes were implied, making it difficult to verify the dose calculated by the authors of the study. Adequate information regarding exposure time to onset and recovery (if any) from the slight neurological functional deficits described was not provided in the report (Maizlish et al. 1987). Other persons occupationally exposed to organophosphorus insecticides, including diazinon, showed no significant change in neurological function, although there was a reduction in serum cholinesterase levels indicating exposure (Stalberg et al. 1978). In contrast, organophosphate poisoning-induced increases in hyperreflexia were reported in workers occupationally exposed to many insecticides, including diazinon. These workers, however, showed no overt signs of poisoning or of cholinergic signs and symptoms after spraying diazinon (Rayner et al. 1972). Two

other insecticide sprayers developed cholinergic symptoms after spraying diazinon. Symptoms included nausea, vomiting, muscle twitching, difficulty breathing, and blurred vision. Serum and erythrocyte cholinesterase activities remained depressed for at least 18 days after exposure (Soliman et al. 1982). In all of these cases of occupational exposure (Rayner et al. 1972; Soliman et al. 1982; Stalberg et al. 1978), no estimate of the exposure level to diazinon was made.

Decreased activity and salivation were noted in Sprague-Dawley rats exposed to 2,330 mg/m^3 diazinon for 4 hours in an inhalation chamber (Holbert 1989). No clinical signs of neurological effects except piloerection were observed in hybrid rats exposed to 0.05, 0.46, 1.57, or 11.6 mg/m^3 diazinon for 3 weeks, 5 days a week for 6 hours a day (Hartman 1990). At study termination, serum cholinesterase activity (a marker for diazinon exposure) was significantly decreased in a dose-related manner in females. Decreases of 20, 27, and 43% were seen at levels of 0.46, 1.57, and 11.6 mg/m^3, respectively. No change was seen at 0.05 mg/m^3. In males, no change was seen at 0.05 or 0.46 mg/m^3, but decreases of 14 and 19% were seen at 1.57 and 11.6 mg/m^3, respectively. Erythrocyte acetylcholinesterase activity (a surrogate marker for neural acetylcholinesterase) was unaffected in females at 0.05 and 0.46 mg/m^3, but was decreased by 10 and 39% at 1.57 and 11.6 mg/m^3, respectively. In males, no change was seen at 0.05, 0.46, or 1.57 mg/m^3, while a decrease of 36% was observed at 11.6 mg/m^3. Brain acetylcholinesterase activity was unchanged in males at all exposure levels, but was decreased in females at 0.05 mg/m^3 (24%), 0.46 mg/m^3 (17%) 1.57 mg/m^3 (20%), and 11.6 mg/m^3 (37%). The decreases in the females at the two lowest exposures are unusual in that no accompanying decrease in erythrocyte acetylcholinesterase was observed. Diazinon exposure had a consistently greater effect on cholinesterase activities in females than in males in this study, although clinical signs of neurological effects besides piloerection were not observed in either sex.

No studies were located regarding organophosphate-induced delayed neurotoxicity (OPIDN) in humans or in animals after inhalation exposure to diazinon.

The highest NOAEL and all LOAEL values from each reliable study for neurological end points in each species and duration category are recorded in Table 2-l and plotted in Figure 2- 1.

2.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after inhalation exposure to diazinon.

2.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after inhalation exposure to diazinon.

2.2.1.7 Genotoxic Effects

Chronic occupational exposure to multiple insecticides, including diazinon, has been associated with an increased incidence of chromosomal aberrations and increased sister chromatid exchanges in peripheral blood lymphocytes as compared with non-exposed populations (De Ferrari et al. 1991; Kiraly et al. 1979; See et al. 1990). Some of these exposures are presumed to be by inhalation. However, it is not possible to attribute the results of these studies to diazinon alone, as workers were exposed to up to 80 different insecticides in unknown amounts for variable durations. Other genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

Several epidemiological studies have reported increased incidence of cancers in humans who were concurrently or sequentially exposed to a number of insecticides, including diazinon. Thus, it is not possible to attribute the increased cancer incidence exclusively to diazinon exposure. Some of the exposure is presumed to have occurred by the inhalation route.

A case-control study. suggested a possible link between family gardening use of diazinon(and other insecticides) and increased incidence of childhood brain cancer (type unspecified). However, this report gave no indication of level, duration, or frequency of exposure to diazinon (or to other insecticides) (Davis et al. 1993). Another case-control study suggested a positive association between an increased incidence of non-Hodgkin’s lymphoma in farmers as compared to non-farmers. The report attributed the increased incidence of lymphomas to handling of organophosphorus insecticides,

including diazinon (Cantor et al. 1992). A third case-control study suggested an association between an increased incidence of multiple myeloma and exposure to high concentrations of insecticides, including diazinon. Actual exposure to diazinon was reported in 2 (0.3%) of the cases and 5 (0.3%) of the controls (Morris et al. 1986).

No studies were located regarding cancer effects in animals after inhalation exposure to diazinon.

2.2.2 Oral Exposure

2.2.2.1 Death

In humans and animals, acute-duration oral exposure to high doses of diazinon induces cholinergic signs and symptoms. With sufficiently high doses of diazinon, extensive edema and hemorrhage in tissues and organs, as well as severe respiratory distress in the victims, have been reported. On some occasions, the respiratory effects progressed to respiratory failure and death preceded by coma. Treatment of test animals with anticholinesterase antagonists such as atropine and pralidoxime (2-PAM) significantly reduced the acute lethality of diazinon in rats, indicating that acute diazinon lethality is primarily attributable to acetylcholinesterase inhibition (Harris et al. 1969).

A summary of autopsy findings of 76 cases of acute diazinon poisoning described cholinergic signs that included: congested, swollen, edematous brain with prominent dural and surface vasculature; livid, congested face; cyanosis; soft flabby heart with conspicuous vasculature on the pericardium and epicardium; cloudy swelling and hyperemia (upon histopathological examination); occasional and scattered petechial and ecchymotic hemorrhage; and occasional brain or spinal hemorrhage. In addition, the victims died with congested respiratory tract, sweating and frothing at the mouth, pulmonary edema and hyperemia, hypostatic congestion, and pneumonia. Generally, the cause of death was respiratory failure and, occasionally, cardiac arrest (Limaye 1966). Other reports of human deaths from diazinon exposure include descriptions of petechial hemorrhages throughout the stomach and gastric mucosa in a diazinon-poisoned 54-year-old female suicide victim who had ingested an estimated 293 mg/kg diazinon (Poklis et al. 1980). Accidental ingestion of an insecticide mixture containing diazinon, parathion, and chlordane resulted in the death of an 8-year-old girl from cardiac and respiratory arrest (De Palma et al. 1970). The estimated dose of diazinon in this case was

20 mg/kg. The toxicity in this case may have been related to the additive effects of diazinon and parathion and/or a possible interaction with chlordane.

A group of 8 children who accidentally became intoxicated by eating oatmeal contaminated with diazinon all recovered (Reichert et al. 1977). A dose could not be determined in this case, but typical neurological signs of diazinon toxicity were observed. Five individuals who intentionally ingested doses of 240-916 mg/kg diazinon recovered after treatment (Klemmer et al. 1978).

The diazinon dose that causes death of experimental animals depends on the form of the test compound (pure, technical, or formulated preparations) as well as on the animal species, sex, and age, and other modifying factors such as diet. It is likely that earlier formulations were more toxic to experimental animals than current ones because of the formation of toxic breakdown products (e.g., sulfotepp) in unstabilized diazinon (Hayes 1982).

In laboratory animal studies, single oral diazinon doses of 50-700 mg/kg to rats resulted in respiratory distress (from pulmonary inflammation and occasional extensive pneumonitis), vascular congestion, and venous stasis in the treated rats. Death generally resulted from respiratory failure that was usually preceded by coma (Boyd and Carsky 1969). A recent study on male Sprague-Dawley rats demonstrated that animals receiving a single oral gavage dose of 2,000 mg/kg diazinon died within 12 hours of dosing, while doses of 500 mg/kg or 1,000 mg/kg did not cause death (Takahashi et al. 1991). The oral LD_{50} dose (lethal dose, 50% kill) of diazinon was determined in white male rats to be 300 mg/kg (Enan et al. 1982).

Oral LD_{50} values for Sherman albino rats were determined to be 108 mg/kg and 76 mg/kg for males and females, respectively, indicating that female rats appear to be more sensitive than male rats (Gaines 1960). When Sherman rats were used, diazinon was less toxic than an earlier report which suggested that males were more sensitive than females, with LD_{50} values of 250 mg/kg and 285 mg/kg for males and females, respectively (Gaines 1969). Another study determined the oral LD_{50} values for male rats for an emulsified solution and wettable paste preparations of diazinon to be 408 and 293 mg/kg, respectively. Males were also found to be the more sensitive sex in a preliminary experiment in this study. Clinical signs of intoxication seen in the dying rats were indicative of cholinergic effects (muscular fibrillation, salivation, lacrimation, incontinence, diarrhea, respiratory distress, hypothermia, prostration, convulsions, gasping, and coma) (Edson and Noakes 1960). An

LD_{50} of 10.9 mg/kg was determined for Red Heavy chickens as part of a delayed neurotoxicity study (Jenkins 1988). The low LD_{50} for chickens reported in this study reflects the relative lack of organophosphate metabolizing enzymes in birds compared to mammals.

The effect of dietary protein on diazinon toxicity was evaluated in a study with male albino Wistar rats. The study concluded that a purified protein test diet (with 26% casein and 59% cornstarch) did not significantly alter the LD_{50} value (415 mg/kg) for diazinon for this species. However, a low protein purified test diet (3.5% casein, 82% cornstarch), lowered the LD_{50} to 215 mg/kg. In addition, this study found that diazinon samples that were time-of-manufacture stabilized (to prevent spontaneous degradation to more toxic monothiotetraethyl pyrophosphate) were less toxic (LD,, value = 466 mg/kg) than samples stabilized after manufacture (LD_{50} value = 271 mg/kg) (Boyd and Carsky 1969). A subsequent study examined the effect of isocaloric diets varied in protein concentration (as casein) from 0% to 81% casein in male albino Wistar rats. The study concluded that while varying dietary protein content from 13 to 3 12% of the normal amount increased acute lethality by 2-fold or less, a protein-free diet resulted in a 7.5-fold increase (although some of this lethality may have resulted from diazinon-induced anorexia and the particular sensitivity of rats on a protein-free diet to starvation). It is also apparent that high or low levels of dietary protein significantly reduce the time to death of diazinon-exposed rats. Clinical signs of diazinon intoxication were similar for all groups and included listlessness, fur soiling, hunched back, piloerection, prostration, exophthalmos, tremors/trembling, dacryorrhea, and shallow respiration (Boyd et al. 1969). A similar study was conducted in male Wistar rats in which the rats were fed defined diets varying in protein content (0, 3.5, 9, 26, and 81% vitamin-free casein) for 28 days prior to exposure to single doses of diazinon in oil by gavage. For rats fed the “normal” 26% casein diet (previously shown to be about equivalent to standard Purina lab chow), the LD_{50} was determined to be 415 mg/kg for a single acute-duration oral exposure. The authors reported observing typical signs of cholinergic stimulation, followed by central nervous system depression. Few signs of protein deficiency were observed when the dietary protein was reduced to 9%, one-third the normal amount. However, diazinon toxicity was increased (i.e., the LD_{50} was reduced) by a factor of 1.8. Even when the protein content was reduced to 3% and signs of protein deficiency were more marked, the LD_{50} for diazinon was reduced by a factor of 1.9. However, rats fed the 0% protein (but normal caloric content) slowly lost body weight, became docile and hypothermic, and with declining water intake increasingly manifested oliguria, aciduria, and occasionally glycosuria. These rats were substantially more susceptible to diazinon toxicity, the LD_{50}

being reduced 7.4-fold. Rats fed 8 1% casein demonstrated various signs of casein intoxication, and the diazinon LD_{50} was reduced by a factor of 2.

Treatment of test animals with anticholinesterase agents such as atropine and 2-PAM significantly reduced the acute lethality of diazinon in rats indicating that acute diazinon lethality is primarily attributable to the inhibition of acetylcholinesterase. Administration of 16 mg/kg atropine intramuscularly, with or without 30 mg/kg pyridine 2-aldoxime methochloride (2-PAM) given either orally or intravenously or both, to female albino rats 10 minutes before diazinon exposure increased the LD_{50} value (294 mg/kg) for diazinon for this species by a factor of 3.2 (with 2-PAM) or 1.7 (without 2-PAM) (Harris et al. 1969).

Deaths have been reported after oral exposure to diazinon in other acute-duration studies. Among Sprague-Dawley rats (10-l 5 of each sex) receiving a single oral gavage dose in corn oil of diazinon of 528 mg/kg, 2 of 15 males and 1 of 15 females died (Chow and Richter 1994). No deaths were reported at 2.2, 132, or 264 mglkg diazinon. Six of 8 New Zealand rabbit dams died when given 30 mg/kg/day diazinon in capsules during gestation days 6-15 (Robens 1969), as did 9 of 22 in the same species receiving 100 mg/kg/day diazinon by gavage during gestation days 6-18 (Harris 1981). No deaths were reported at 7 mg/kg/day during gestation days 6-15 (Robens 1969), or at 7 and 25 mg/kg/day during gestation days 6-18 (Harris 1981) in New Zealand rabbits. No deaths were reported in pregnant CD-l rats receiving 10, 20, or 100 mg/kg/day diazinon during gestation days 6-15 (Infurna et al. 1985).

Intermediate-duration oral administration of 10 or 20 mg/kg/day diazinon dissolved in corn oil in gelatin capsules for 8 months to Beagle dogs (3 males and 3 females per group) resulted in mortality (1 of 3 males and 1 of 3 females at 20 mg/kg). Toxic signs, which were not consistent in all the dogs at a given dose, did not show a dose-response relationship. Generally, female dogs were less sensitive to diazinon toxicity than male dogs (Earl et al. 1971). In another study in which Hormel-Hanford miniature swine of both sexes were administered daily oral doses of diazinon dissolved in oil in capsules for up to 8 months resulted in mortality (100% in males and 67% in females) in 12-38 days of treatment at the highest dose tested (10 mg/kg/day) (Earl et al. 1971).

No deaths were reported in Sprague-Dawley rats (groups of 10 of each sex) receiving up to 183.2 mg/kg/day diazinon in feed for 6 weeks or up to 212 mg/kg/day (groups of 15 of each sex) for

13 weeks (Singh 1988) or in Beagle dogs (groups of 4 of each sex) receiving up to 15.99 mg/kg/day in feed for 4 weeks or up to 11.6 mg/kg/day diazinon for 13 weeks (Barnes 1988). Survival rates were similar to controls in Sprague-Dawley rats receiving up to 12 mg/kg/day diazinon in feed for 98 weeks (Kirchner et al. 1991).

The LD_{50} values and doses associated with death in each species and duration category are shown in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

No studies were located regarding musculoskeletal, dermal or body weight effects in humans after oral diazinon exposure. No information on musculoskeletal or dermal effects in animals after oral exposure to diazinon was located. Autopsy findings in human acute diazinon poisonings and laboratory animal lethality studies, as well as findings from other human and laboratory animal non-lethal oral exposures, included respiratory impairment, cardiovascular, gastrointestinal, hematological, and endocrine (pancreas) effects. These effects were largely derived from cholinergic responses that stemmed from inhibition of acetylcholinesterase by high doses of organophosphate (diazinon) in humans and laboratory animals.

The highest NOAEL value and all LOAEL values for adverse systemic effects in each reliable study for each species and duration category are shown in Table 2-2 and plotted in Figure 2-2.

Respiratory Effects. Respiratory distress, as a component of the spectrum of the symptoms of cholinergic reaction resulting from acetylcholinesterase inhibition, was reported in several human acute poisoning incidents and laboratory animal evaluation following oral diazinon exposure. In humans, acute-duration oral exposure to high doses of diazinon causes pulmonary distress with signs that include congested respiratory tract, copious airway secretions, and pulmonary edema (Balani et al. 1968; Hata et al, 1986; Kabrawala et al. 1965). An 18% incidence of pulmonary edema-was found in diazinon-poisoned patients (Limaye 1966; Shankar 1967). An autopsy report of a diazinon-poisoned 54-year-old female suicide victim described heavy and congested (edematous) lungs (Poklis et al. 1980). Tachypnea and cyanosis were observed in a male who intentionally ingested 240 mg/kg diazinon and in a female who ingested 509 mg/kg (Klemmer et al. 1978). Diazinon treatment also resulted in signs of respiratory effects in laboratory animals. Single oral diazinon doses of

Table 2-2. Levels of Significant Exposure to Diazinon - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	ACUTE EXPOSURE						
	Death						
1	Human	once (IN)				293 F (death)	Poklis et al. 1980
2	Rat (Wistar)	once (GO)				466 M (LD_{50})	Boyd and Carsky 1969
3	Rat (Wistar albino)	once (GO)				415 M (LD_{50})	Boyd et al. 1969
4	Rat (Sprague-Dawley)	once (GO)				528 (2/15 males and 1/15 females died)	Chow and Richter 1994
5	Rat (Wistar albino)	once (GW)				408 M (LD_{50} : emulsified solution) 293 M (LD_{50} : wettable paste)	Edson and Noakes 1960
6	Rat (white)	once (GO)				300 M (LD_{50})	Enan et al. 1982
7	Rat (Sherman)	once (GO)				108 M (LD_{50}) 76 F (LD_{50})	Gaines 1960
8	Rat (Sherman)	once (GO)				250 M (LD_{50}) 285 F (LD_{50})	Gaines 1969
9	Rat (albino)	once (GO)				294 F (LD_{50})	Harris et al. 1969

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
10	Rat (Sprague-Dawley)	once (GW)				2000 M (3/3 died)	Takahashi et al. 1991
11	Rabbit (New Zealand)	Gd 6-18 once/d (G)				100 F (9/22 died)	Harris 1981
12	Rabbit (New Zealand)	Gd 5-15 1x/d (C)				30 F (6/8 died)	Robens 1969
13	Chicken (Red Heavy)	once (GO)				10.9 F (LD_{50})	Jenkins 1988
	Systemic						
14	Human	once (IN)	Resp			240 M (tachypnea, cyanosis) 509 F	Klemmer at al. 1978
			Cardio			240 M (bradycardia, tachycardia) 509 F	
			Hemato	240 M 509 F			
			Metabolic			240 M (metabolic acidosis) 509 F	
15	Human	once (IN)	Resp			293 F (heavily congested lungs)	Poklis et al. 1980
			Gastro			293 F (petechial hemorrhages throughout the stomach and gastric mucosa)	

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
16	Rat (Sprague-Dawley)	once (GO)	Hemato	528			Chow and Richter 1994
			Ocular		528 (chromodacryorrhea)		
			Bd Wt	132 M 528 F		264 M (25% decrease in weight gain)	
17	Rat (Wistar)	7 d ad lib (F)	Bd Wt	0.21			Davies and Holub 1980b
18	Rat (CD-1)	Gd 6-15 once/d (G)	Bd Wt	20 F	100 F (5.5-9.6% decrease in maternal weight, 26-30% decrease in feed consumption)		Infurna et al. 1985
19	Rat (Sprague-Dawley)	once (GW)	Hemato		4.4 M (reduced platelet count, altered coagulation factor activities)		Lox 1983
20	Rat (Sprague-Dawley)	14 d ad libitum (W)	Hemato		52 F (reduced hematocrit, altered clotting factor activities)		Lox 1987
			Bd Wt	52			
21	Rat (Sprague-Dawley)	once (G)	Hepatic		300 (reduced hepatic cytochrome P-450, aniline hydroxylase, aminopyrine N-demethylase)		Mihara et al. 1981

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
22	Rabbit (New Zealand)	Gd 6-18 once/d (G)	Resp	100 F			Harris 1981
			Cardio	100 F			
			Gastro	25 F		100 F (7/9 stomach mucosal hemorrhage, congestion and erosion)	
			Hepatic	100 F			
			Renal	100 F			
			Bd Wt	100 F			
23	Chicken (Red Heavy)	2x days 0, 21 (GO)	Bd Wt	11.3 F			Jenkins 1988
	Neurological						
24	Human	once (IN)				240 M (stupor, profuse diaphoresis, coma) 509 F	Klemmer at al. 1978
25	Human	once (IN)				293 F (petechial hemorrhages throughout the brain)	Poklis et al. 1980
26	Rat (Sprague-Dawley)	once (GO)		2.2		132 (82% decrease in erythrocyte AChE, ataxia, alterations in functional observation battery tests 9-11 hrs post-dosing)	Chow and Richter 1994
27	Rat (Wistar)	7 d ad lib (F)		0.21			Davies and Holub 1980b

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
28	Rat (Wistar albino)	once (GW)				300 M (erythrocyte AChE decreased 89% 24 hrs after exposure)	Edson and Noakes 1960
29	Rat (albino)	once (GO)				235 F (78% decrease in brain AChE)	Harris et al. 1969
30	Rat (Sprague-Dawley)	once (GW)		500 M		1000 M (fasciculations, twitches, convulsions, Straub tail reflex)	Takahashi et al. 1991
31	Hamster (Golden Syrian)	Gd 6, 7 and/or 8 1 x/d (GO)			0.125 F (diarrhea, salivation, incoordination)		Robens 1969
32	Rabbit (New Zealand)	Gd 6-18 once/d (G)		25 F		100 F (tremors, convulsion)	Harris 1981
33	Rabbit (New Zealand)	Gd 5-15 1 x/d (C)		7 F		30 F (ataxia)	Robens 1969
34	Chicken (Red Heavy)	2 x on days 0, 21 (GO)		11.3 F			Jenkins 1988
	Reproductive						
35	Rat (CD-1)	Gd 6-15 once/d (G)		100 F			Infurna et al. 1985

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference
36	Rabbit (New Zealand)	Gd 6-18 once/d (G)		100 F				Harris 1981
	Developmental							
37	Rat (CD-1)	Gd 6-15 once/d (G)		20		100	(increased incidence of rudimentary ribs at T-14 in fetuses)	Infurna et al. 1985
38	Hamster (Golden Syrian)	Gd 6, 7 and/or 8 1 x/d (GO)		0.25				Robens 1969
39	Rabbit (New Zealand)	Gd 6-18 once/d (G)		100				Harris 1981
40	Rabbit (New Zealand)	Gd 5-15 1 x/d (C)		30				Robens 1969

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	INTERMEDIATE EXPOSURE						
	Death						
41	Dog (Beagle)	8 mo 1 x/d (C)				10 (3/3 males and 2/3 females died)	Earl et al. 1971
42	Pig (Hormel-Hanford)	8 mo 1 x/d (C)				10 (3/3 males and 2/3 females died)	Earl et al. 1971
	Systemic						
43	Rat (Wistar)	7-28 wk 2 x/wk (G)	Hepatic		0.5 M (lipid vacuolation)		Anthony et al. 1986
			Bd Wt		0.5 M (10% reduction in body weight gain)		
44	Rat (Wistar)	35-92 d ad lib (F)	Bd Wt	1.35			Davies and Holub 1980a
45	Rat (Wistar)	30 d ad lib (F)	Bd Wt	2.86			Davies and Holub 1980b
46	Rat (Wistar albino)	16 wk ad lib (F)	Hepatic	11.7 M			Edson and Noakes 1960
			Renal	11.7 M			
			Bd Wt	11.7 M			

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
47	Rat (white)	4 wk ad lib (F)	Hepatic		30 M (reduced serum beta-lipoproteins, increased alanine aminotransferase, aspartate amino-transferase, gamma-glutamyl transferase, lactate		Enan et al. 1982
48	Rat (Sprague-Dawley)	6 mo ad lib (W)	Hemato	0.18 F			Lox and Davis 1983
			Hepatic	0.18 F			
			Bd Wt	0.18 F			

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
49	Rat (Sprague-Dawley)	13 wk 7 d/wk ad lib (F)	Resp	168 M 212 F			Singh 1988
			Cardio	168 M 212 F			
			Gastro	19 M 15 F	168 M (soft stools) 212 F (soft stools)		
			Hemato	168 M 19 F		212 F (decreased hemoglobin and hematocrit; increase in reticulocytes)	
			Hepatic	168 M 19 F	212 F (increase in relative and absolute liver weight, minimal centrolobular hepatocellular hypertrophy)		
			Renal	168 M 212 F			
			Endocr	168 M 212 F			
			Ocular	168 M 212 F			
			Bd Wt	168 M 212 F			
50	Rat (Sprague-Dawley)	6 wk 7 d/wk ad lib (F)	Gastro	0.2 M 9.4 F	8.4 M (soft stools) 183.2 F (soft stools)		Singh 1988
			Bd Wt	8.4	150.8 M (15% decrease in body weight)		

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
51	Dog (Beagle)	13 wk 7 d/wk (F)	Resp	11.6			Barnes et al. 1988
			Cardio	11.6			
			Gastro	11.6			
			Hemato	11.6			
			Hepatic	11.6			
			Renal	11.6			
			Endocr	5.6 M 11.6 F		10.9 M (atrophy of pancreatic acini)	
			Ocular	11.6			
			Bd Wt	5.9 M 0.21 F		10.9 M (34% decreased weight gain 5.6 F in males, 33% in females)	
52	Dog (Beagle)	4 wk 7d/wk (F)	Hemato	15.99			Barnes et al. 1988
			Hepatic	15.99			
			Renal	15.99			
			Bd Wt	0.8	14.68 M (weight loss)	15.99 F (emaciation-20% wt loss)	

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
53	Dog (Beagle)	8 mo 1 x/d (C)	Cardio	5 M 20 F	10 M (no pericardial fat, cord-like heart vessels)		Earl et al. 1971
			Gastro	5	10 M (duodenal wall thickening)	20 (duodenal and stomach ruptures)	
			Hemato	10 F		10 M (peripheral anemia; bone 20 F marrow hypocellularity, increased myeloid element content, reticulocytopenia)	
			Hepatic	2.5	5 (markedly elevated serum aspartate aminotransferase and ornithine carbamyl transferase)	10 M (yellow, fatty liver; parenchymal atrophy, hepatocyte dissociation; moderate cirrhosis, focal necrosis, fibrous infiltration, elevated serum lactate dehydrogenase)	
			Renal	5 M 10 F		10 M (localized chronic nephritis, tubular atrophy, glomeruli with fibrous infiltration)	
			Endocr	5		10 M (pancreatic atrophy and interstitial fibrosis)	
			Bd Wt	5 M 10 F	10 M (significant weight loss) 20 F (significant weight loss)		

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference
54	Pig (Hormel-Hanford)	8 mo 1 x/d (C)	Gastro	1.25	2.5	(edema and serosal seepage in the ileum)	10	(jejunal edema, localized mucosal erosion into intestinal muscle layers with marked serosal seepage; duodenal ulceration)	Earl et al. 1971
			Hemato	2.5			5	(occasional transient peripheral anemia, reticulocytopenia, bone marrow hypocellularity, increased myeloid element content)	
			Hepatic		1.25	(slight inflammation, occasional lobular congestion)	2.5 5	(interlobular connective tissue thickening, degenerative hepatocytes, hepatic hemorrhage)	
Immunological/Lymphoreticular									
55	Rat (Sprague-Dawley)	13 wk 7 d/wk ad lib (F)		168 M 212 F					Singh 1988
56	Dog (Beagle)	13 wk 7 d/wk (F)		11.6					Barnes et al. 1988

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	Neurological						
57	Rat (Wistar)	35-92 d ad lib (F)		1.35			Davies and Holub 1980a
58	Rat (Wistar)	30 d ad lib (F)			2.86 (58% decrease in erythrocyte AChE)		Davies and Holub 1980b
59	Rat (Wistar albino)	16 wk ad lib (F)		0.44 M	2.26 M (46% decrease in erythrocyte AChE)	11.7 M (79% decrease in erythrocyte AChE)	Edson and Noakes 1960
60	Rat (Sprague-Dawley)	13 wk 7 d/wk ad lib (F)		0.4	15 M (27% decrease in erythrocyte AChE)		Singh 1988
61	Rat (Sprague-Dawley)	6 wk 7 d/wk (F)		0.2	8.4 M (21% decrease in erythrocyte AChE) 9.4 F (24% decrease in brain AChE)	150.8 M (58% decrease in brain AChE in males, 61% decrease in females) 183.2 F	Singh 1988
62	Dog (Beagle)	13 wk 7 d/wk (F)		0.021 [b]	5.9 (31% decrease in erythrocyte and brain AChE)		Barnes et al. 1988
63	Dog (Beagle)	4 wk 7 d/wk (F)		0.082	14.68 (30% decrease in erythrocyte AChE, 44% decrease in brain AChE, emesis)		Barnes et al. 1988

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
64	Dog (Beagle)	8 mo 1 x/d (C)		5	10 (fasciculation, diarrhea, emesis)		Earl et al. 1971
65	Dog (mixed breed)	12 wk 7 d/wk (F)		0.019	1.9 (55% decrease in erythrocyte AChE)		Williams et al. 1959
66	Pig (Hormel-Hanford)	8 mo 1 x/d (C)		1.25	2.5 (emesis, diarrhea, fasciculations)		Earl et al. 1971
	Reproductive						
67	Rat (Sprague-Dawley)	60 d ad libitum (F)		0.05			Green 1970
68	Rat (Sprague-Dawley)	13 wk 7 d/wk ad lib (F)		168 M 212 F			Singh 1988
69	Mouse (Hybrid)	Gd 1-18 1 x/d (F)			0.18 (14% reduced maternal weight gain, 20% reduced litter size)		Spyker and Avery 1977
70	Dog (Beagle)	13 wk 7 d/wk (F)		11.6			Barnes et al. 1988
71	Dog (Beagle)	8 mo 1 x/d (C)		5M		10 M (testicular atrophy, aspermatogenesis)	Earl et al. 1971

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
Developmental								
72	Mouse (Hybrid)	Gd 1-18 1 x/d (F)		0.18		9	(significantly reduced early weight gain by pups, increased mortality at ppd 28)	Barnett et al. 1980
73	Mouse (Hybrid)	Gd 1-18 1 x/d (F)				0.18	(neuromuscular coordination deficits, reduced litter size, delayed contact placing and sexual maturity)	Spyker and Avery 1977

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	CHRONIC EXPOSURE						
	Systemic						
74	Rat (Sprague-Dawley)	98 wks or 52 wks (F)	Resp	10 M 12 F			Kirchner et al. 1991
			Cardio	10 M 12 F			
			Gastro	10 M 12 F			
			Hemato	10 M 12 F			
			Musc/skel	10 M 12 F			
			Hepatic	10 M 12 F			
			Renal	10 M 12 F			
			Endocr	10 M 12 F			
			Dermal	10 M 12 F			
			Ocular	10 M 12 F			
			Bd Wt	10 M 12 F			
			Metabolic	10 M 12 F			

Table 2-2. Levels of Significant Exposure to Diazinon - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	Immunological/Lymphoreticular						
75	Rat (Sprague-Dawley)	98 wks or 52 wks (F)		10 M 12 F			Kirchner et al. 1991
	Neurological						
76	Rat (Sprague-Dawley)	98 wks or 52 wks (F)		0.06 M 0.07 F	5 M (24% decrease in brain 6 F AChE in males, 29% in females)		Kirchner et al. 1991
	Reproductive						
77	Rat (Sprague-Dawley)	98 wks or 52 wks (F)		10 M 12 F			Kirchner et al. 1991

[a]The number corresponds to entries in Figure 2-2.

[b]Used to derive an intermediate-duration Minimal Risk Level (MRL) of 0.0002 mg/kg/day based on the NOAEL of 0.021 mg/kg/day for brain acetylcholinesterase inhibition, using an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

AChE = acetylcholinesterase; ad lib = ad libitum; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = female; (F) = feed; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; gen = generation; (GO) = gavage in oil; (GW) = gavage in water; Hemato= hematological; (IN) = ingestion; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observable-adverse-effect level; M = male; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; Resp = respiratory; (W) = water; wk = week(s); x = times

Figure 2-2. Levels of Significant Exposure to Diazinon - Oral

Acute (≤14 days)

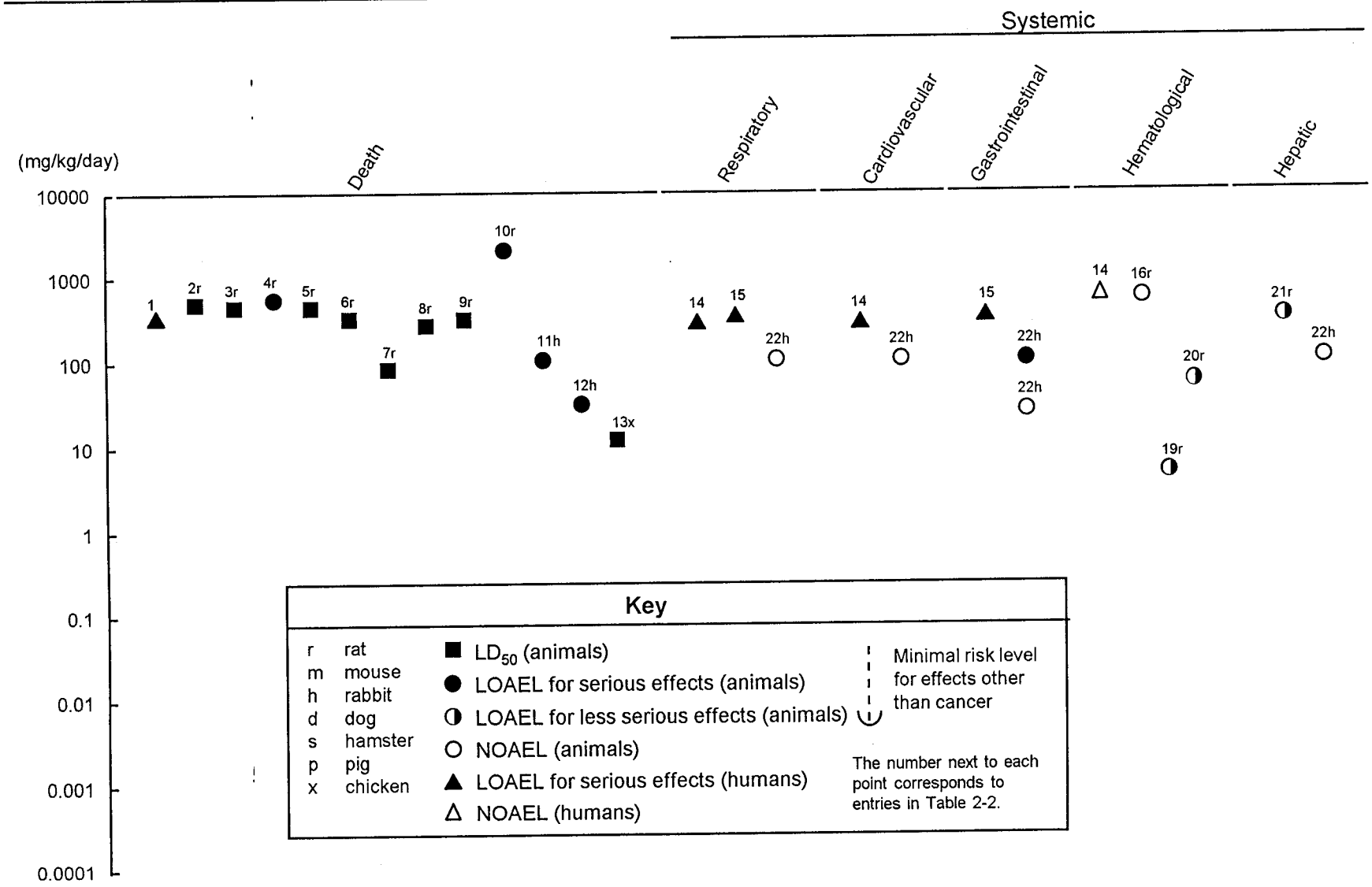


Figure 2-2. Levels of Significant Exposure to Diazinon - Oral (cont.)

Acute (≤14 days)

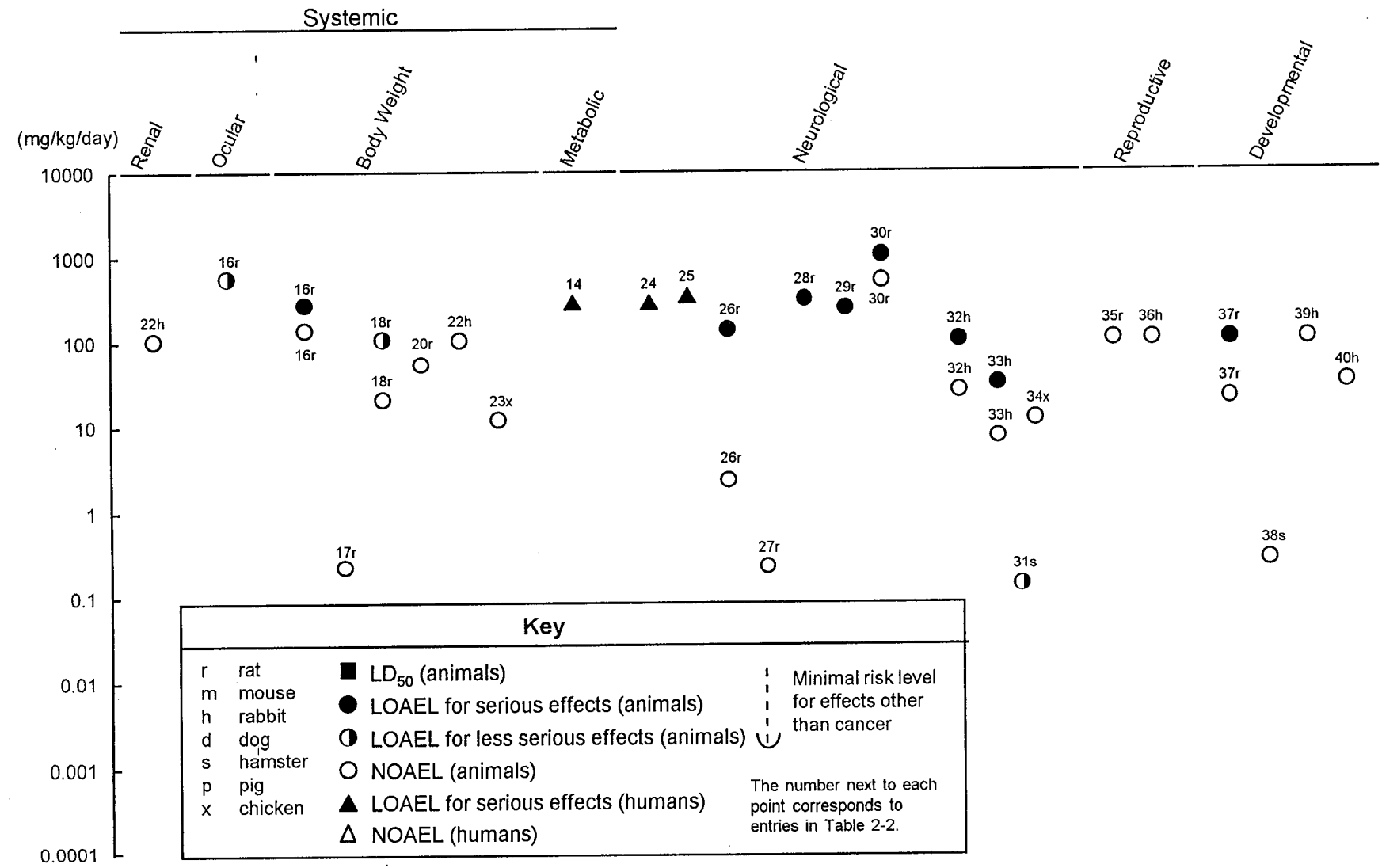


Figure 2-2. Levels of Significant Exposure to Diazinon - Oral (cont.)

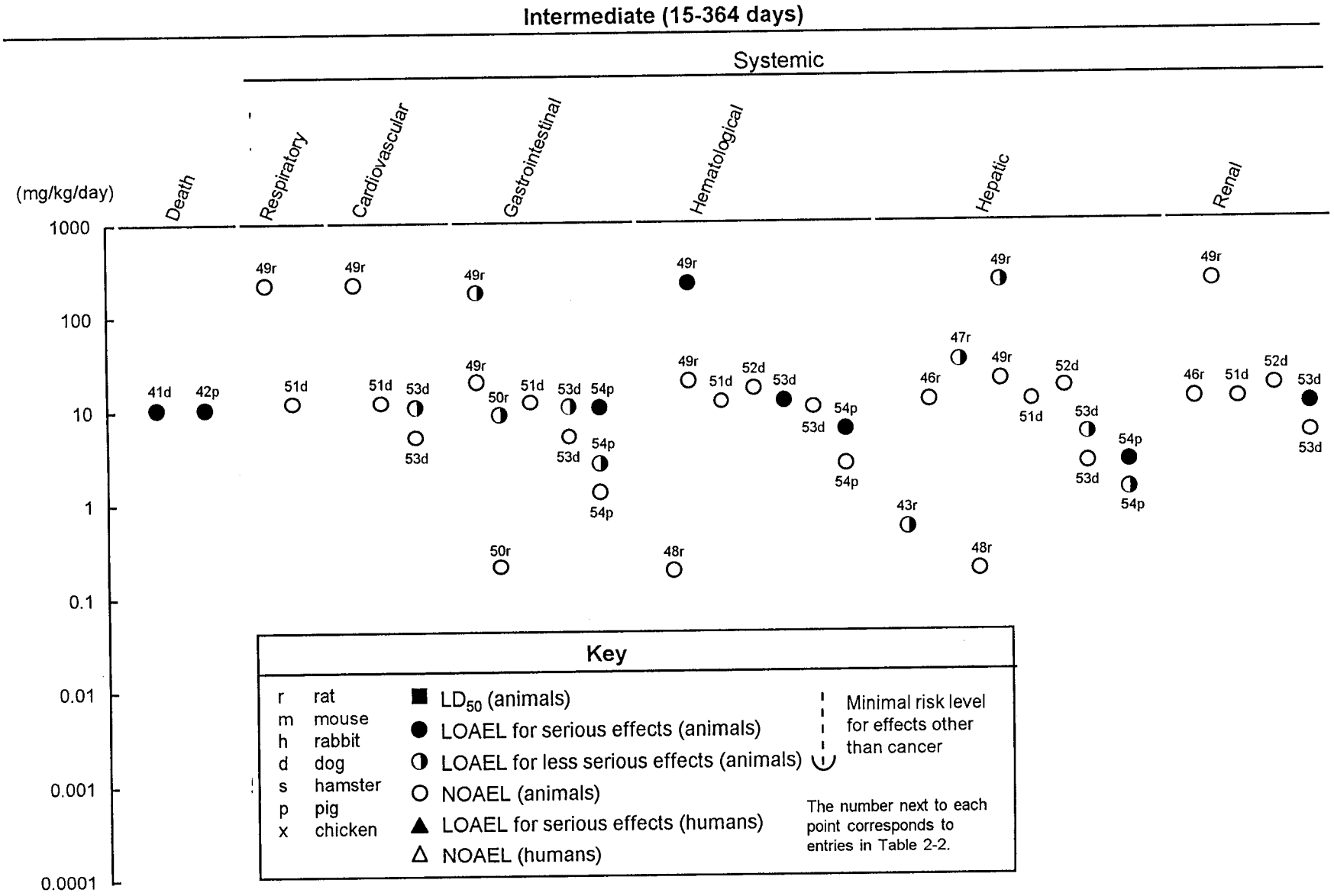


Figure 2-2. Levels of Significant Exposure to Diazinon - Oral (cont.)

Intermediate (15-364 days)

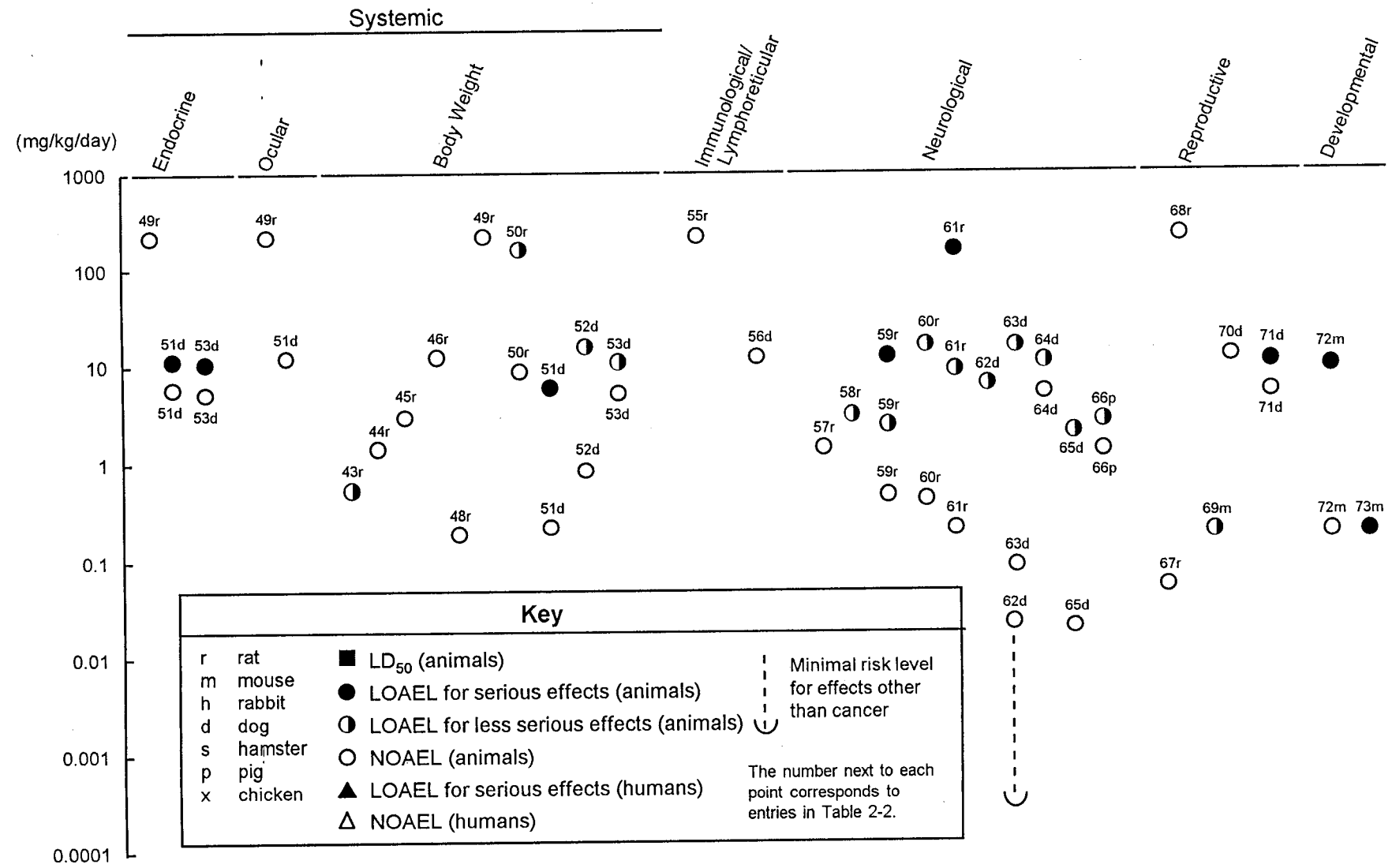
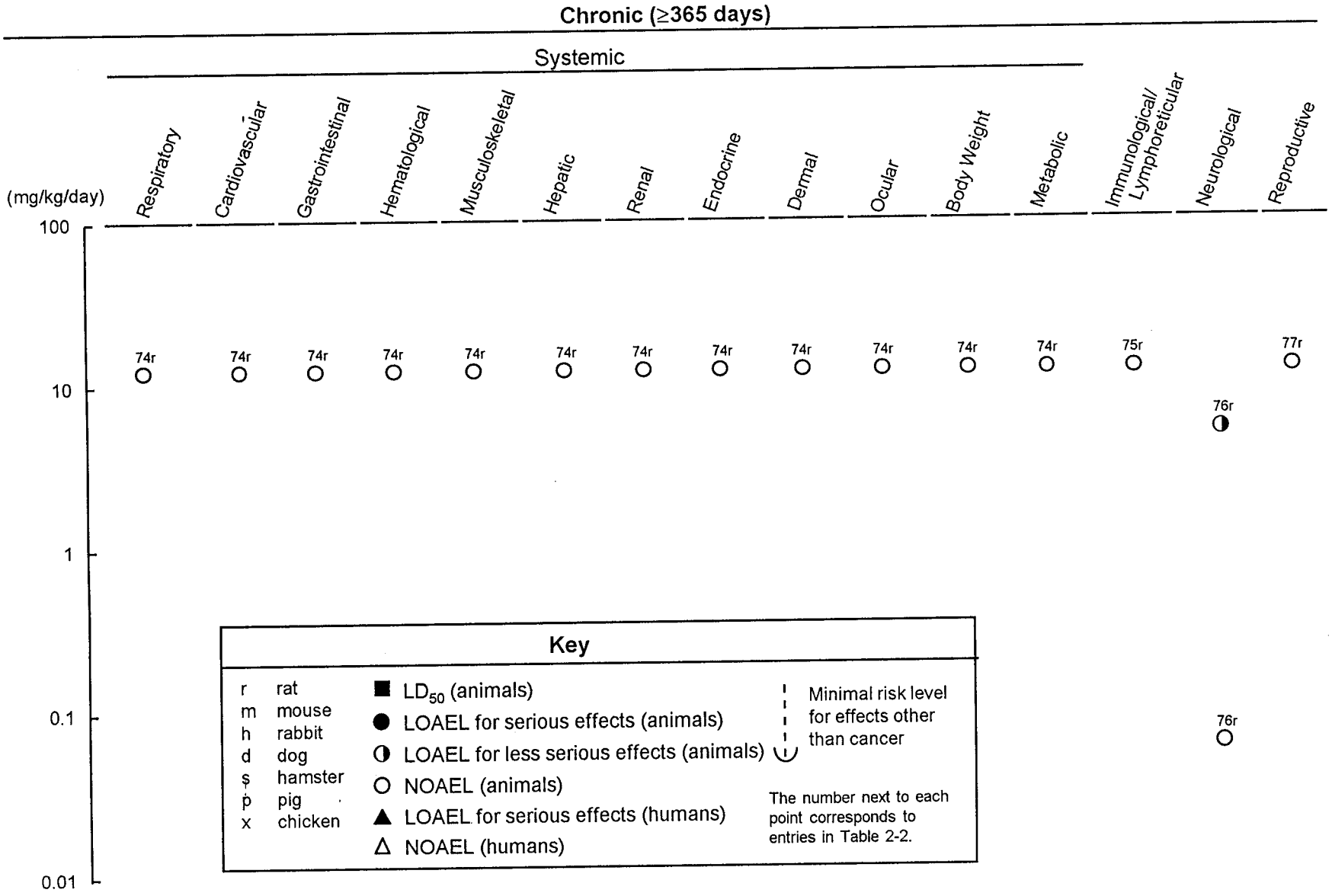


Figure 2-2. Levels of Significant Exposure to Diazinon - Oral (cont.)



50-700 mg/kg to rats resulted in respiratory distress from pulmonary inflammation, vascular congestion, venous stasis, and occasional extensive pneumonitis in the treated rats. Death generally resulted from respiratory failure that was usually preceded by coma (Boyd and Carsky 1969). Dyspnea was observed in male Sprague-Dawley rats given a single gavage dose of 264 mg/kg diazinon and impaired respiration was observed in females receiving a dose of 528 mg/kg (Chow and Richter 1994).

No gross or histological evidence of treatment-related damage to the lungs after oral exposure to diazinon was observed in New Zealand rabbit dams receiving up to 100 mg/kg/day diazinon during gestation days 6-18 (Harris 1981), in Sprague-Dawley rats (groups of 15 of each sex) receiving up to 212 mg/kg/day diazinon in feed for 13 weeks (Singh 1988) or up to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991), or in Beagle dogs (groups of 4 of each sex) receiving up to 11.6 mg/kg/day diazinon for 13 weeks (Barnes 1988).

Cardiovascular Effects. Acute-duration oral, lethal human exposure to diazinon resulted in extensive congestion of the heart and blood vessels as reported in a summary of autopsy findings of 76 cases of acute diazinon poisoning which described cardiovascular signs that included: livid, congested face; soft flabby heart with conspicuous vasculature on the pericardium and epicardium; occasional and scattered petechial/ecchymotic hemorrhage; and cloudy swelling and hyperemia (upon histopathological examination) (Limaye 1966). In a case study of 25 persons that attempted suicide by ingesting diazinon, some patients showed hypertension and peripheral circulatory failure (Kabrawala et al. 1965). Other cardiovascular signs reported after acute oral exposure to high doses of diazinon in humans include tachycardia (Kabrawala et al. 1965; Klemmer et al. 1978; Shankar 1967), hypertension (Balani et al. 1968; Hata et al. 1986), and bradycardia (Hata et al. 1986; Klemmer et al. 1978).

One male dog given 10 mg/kg/day diazinon for 8 months exhibited an absence of pericardial fat on the heart, as well as a cord-like appearance of the heart vessels (Earl et al. 1971). Two other dogs, given 10 or 20 mg/kg/day diazinon, exhibited markedly elevated serum lactate dehydrogenase (LDH). This is a nonspecific response that may be suggestive of either cardiac or skeletal muscle damage or some other unknown pathology. Pallor was reported in male Sprague-Dawley rats receiving a single oral dose of 132 mg/kg diazinon (Chow and Richter 1994).

No gross or histological evidence of treatment-related damage to the heart after oral exposure to diazinon was observed in New Zealand rabbit dams receiving up to 100 mg/kg/day diazinon during gestation days 6-18 (Harris 1981), in Sprague-Dawley rats (groups of 1.5 of each sex) receiving up to 212 mg/kg/day diazinon in feed for 13 weeks (Singh 1988), or up to 12 mg/kg/day diazinon in feed for 98 weeks (Kirchner et al. 1991), or in Beagle dogs (groups of 4 of each sex) receiving up to 11.6 mg/kg/day diazinon for 13 weeks (Barnes 1988).

Gastrointestinal Effects. A summary of autopsy findings of 76 cases of acute diazinon poisoning described gastrointestinal signs that include: dark, blood-stained stomach contents; congested stomach mucosa with submucosal petechial hemorrhage; and occasional erosion and ulceration (Limaye 1966). Petechial hemorrhages throughout the stomach and gastric mucosa were revealed in the autopsy report of a diazinon-poisoned 54-year-old female suicide victim who had ingested an estimated 293 mgfkg diazinon (Poklis et al. 1980). Other signs of gastrointestinal toxicity seen in humans after acute exposure to high doses of diazinon include nausea, diarrhea and vomiting (Balani et al. 1968; Klemmer et al. 1978), and abdominal pain (Balani et al. 1968). A 16-year-old female who drank an estimated 1.5 mg/kg of a diazinon formulation (Tik-20) developed pancreatitis after being treated for cholinergic manifestations. The pancreatic effects may well have been secondary to the diazinoninduced cholinergic manifestations (Dagli et al. 1981). Acute pancreatitis was also found in two children poisoned with diazinon (Weizman and Sofer 1992).

In male albino Wistar rats exposed to high acute doses of diazinon prior to death, lamina propria of the small intestine were congested, and occasional small areas of hemorrhage and necrosis at the mouth of gastric glands were observed. The digestive tract was dehydrated with small increases in organ wet weight except for the cecum, whose wet weight declined approximately 32%. Other effects included pyloric stomach ulceration and inflammation of the small intestine and cecum (Boyd and Carsky 1969). Similar effects were seen in an intermediate exposure of Beagle dogs given doses of diazinon for 8 months. Marked edematous thickening of the intestinal wall was observed in 5 of 6 dogs at the 20 mg/kg/day dose with one developing a duodenal rupture and subsequent.peritonitis, and another a rupture of the pyloric portion of the stomach. At the 10 mg/kg/day dose, the duodenal wall thickening was observed only in the male dog that exhibited weight loss and other gross pathological changes. Elevated serum amylase levels were also found in dogs of both sexes at the 10 mg/kg/day dose but apparently did not correlate with observable pancreatic pathology with the exception of one male dog. Either congestion or hemorrhage (or both) of the small intestines and

colon was present in varying degrees among dogs receiving 5-100 mg/kg/day diazinon for various time periods in a preliminary dose-range study. Apparently, many of the effects described were not found uniformly in all of the dogs at a given dose, and a clear dose-response relationship was not always present (Earl et al. 1971). Intermediate-duration treatment of Hormel-Hanford miniature pigs with daily doses of 1.25-10 mg/kg/day for 8 months resulted in injury to the gastrointestinal tract. At 10 mg/kg/day, 4 of 5 pigs which died had edematous thickening of the walls of the jejunum, 3 of 5 had ulcer formation in the duodenum, and one had localized mucosal erosion into the muscular layer with serosal seepage throughout the intestines. One pig at each of the 5.0 and 2.5 mg/kg/day doses displayed edema of the jejunum, with serosal seepage of the ileum also observed at the lower dose. Histopathologically, slight thickening of the serosa, occasional focal hyperemia, and outer muscle hemorrhaging were observed in the intestines of swine exposed to 10 or 5 mg/kg/day. Abdominal ascites that clotted on exposure to air was reported without further description for one pig exposed to 2.5 mg/kg/day. This animal also suffered intestinal edema and serosal seepage, liver toxicity, and death on day 141 (Earl et al. 1971).

Stomach mucosal hemorrhage, congestion, and erosion were observed in 7 of 9 New Zealand rabbit dams that died while receiving 100 mg/kg/day diazinon during gestation days 6-18 (Harris 1981). No signs of gastrointestinal toxicity were seen in dams treated at 7 or 25 mg/kg/day. Diarrhea was observed in male Sprague-Dawley rats receiving a single oral dose of 528 mg/kg diazinon, but not in females receiving the same dose (Chow and Richter 1994). Soft stools were observed in male Sprague-Dawley rats receiving 8.4 mgikg/day diazinon in feed for 6 weeks and in females receiving 183.2 mg/kg/day (Singh 1988), as well as males receiving 168 mg/kg/day diazinon in feed for 13 weeks and in females receiving 212 mg/kg/day (Singh 1988). Emesis was reported in male and female Beagle dogs receiving 14.68 mg/kg/day diazinon in feed for 4 weeks (Barnes 1988). Emesis, bloody feces, and diarrhea were observed in Beagle dogs receiving up to 11.6 mg/kg/day diazinon in feed for 13 weeks. These signs were not dose-related and were considered by the authors to be unrelated to treatment (Barnes 1988).

No histological evidence of treatment-related damage to gastrointestinal tissues was found in Sprague-Dawley rats receiving up to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991), or up to 212 mg/kg/day for 13 weeks (Singh 1988). Similar results were reported in Beagle dogs receiving up to 11.6 mg/kg/day diazinon over a 13-week period (Barnes 1988).

Hematological Effects. A report on 5 individuals (3 males, 2 females) who intentionally ingested 60-180 mL of 25% diazinon solution (estimated to deliver a dose of 240-400 mg/kg for males and 509-986 mg/kg for females) found that leucocyte counts (3,700, 95% polymorphonuclear), hemoglobin (16.3 g), and hematocrit (47) were all within normal ranges (Klemmer et al. 1978).

In laboratory animal studies, the hematological effects of a single oral dose of 4.4 mg/kg diazinon was studied in Sprague-Dawley rats 2 hours after treatment. While diazinon exposure did not significantly alter hematocrit or factor VII activity, platelet count was significantly ($p<0.05$) reduced when compared with pre-exposure values ($694x10^3/mm^3$ as compared to $856x10^3/mm^3$). Similarly, small (6-14%) but significant (<0.05) changes were observed in activities of the remaining clotting factors; fibrinogen activity was reduced, while prothrombin, partial thromboplastin, factor II, factor V, and factor X activities were increased. Since fibrinogen and factors II, V, VII, and X are synthesized in the liver, the associated alterations may reflect hepatic effects of diazinon exposure. The data indicate an overall diazinon-induced condition of hyper coagulability that, considered together with observations from other studies of various haemorrhagia, may suggest that diazinon might affect hemostasis in general (Lox 1983). Other rats were exposed for 14 days to 52 mg/kg/day diazinon in drinking water and monitored for hematocrit and platelet count, and various clotting factor times were monitored (prothrombin, partial thromboplastin, fibrinogen, and factors II, V, VII, X, and XII). Immediately after treatment, increased times for prothrombin, partial thromboplastin, and fibrinogen suggest an overall state of hypocoagulability, despite no consistent pattern for the other factors and parameters (decreased for VII and XII, no changes for II, V, VII, and X, or in hematocrit and platelet count). One week after treatment, partial thromboplastin time was shortened (indicating intrinsic pathway activation), as were the clotting times for factors VIII, X, and XII, although that for II was lengthened. Overall, this suggests a hyper coagulability of the intrinsic pathway. Also, hematocrit was decreased. These alterations may reflect a time-course in hepatic damage (at least for II, VII, X, and fibrinogen which are of liver origin) (Lox 1987). A group of 24 rats exposed to a lower dose of approximately 0.18 mg/kg/day diazinon in drinking water for 6 months showed no changes compared with controls in the clotting activities associated with prothrombin, partial thromboplastin, fibrinogen, or the coagulation factors II, V, VII, and X (Lox and Davies 1983). In another study, one dog treated with 20 mg/kg/day diazinon showed marked reductions in peripheral red blood cells, hematocrit, and hemoglobin. Its myeloid/erythroid (M/E) bone marrow ratio was evidently within the normal range. At the 20 mg/kg/day dose, all the dogs displayed greatly elevated M/E ratios (114-l 83/l as opposed to 1. l-l .9/l for controls) with slight to moderate bone marrow hypocellularity, and a pronounced

reticulocytopenia in 2 dogs (one male, one female) (Earl et al. 1971). Intermediate-duration treatment of Hormel-Hanford miniature pigs with daily doses of diazinon resulted in hematological effects. Three of 6 pigs exposed to 5.0 mg/kg/day diazinon showed a transient drop in red blood cells, hematocrit, and hemoglobin content, but no indication of peripheral anemia. No peripheral anemia was present in any of 5 pigs in the 10 mg/kg/day group, but all the pigs exhibited reticulocytopenia, with 3 displaying elevated M/E ratios (Earl et al. 1971).

Hematological parameters were normal in Sprague-Dawley rats (groups of lo-15 of each sex) receiving a single oral gavage dose of up to 528 mg/kg diazinon and examined 14 days later (Chow and Richter 1994). Decreased hemoglobin and hematocrit along with an increase in reticulocytes were observed in female Sprague-Dawley rats receiving 212 mgikg/day diazinon in feed for 13 weeks. Hematological parameters were normal in female rats receiving up to 19 mg/kg/day and in males receiving up to 168 mg/kg/day (Singh 1988). No changes in hematological parameters were observed in Beagle dogs (groups of 4 of each sex) receiving up to 11.6 mgikg/day diazinon in feed (Barnes 1988), or in Sprague-Dawley rats receiving up to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991).

Hepatic Effects. A summary of autopsy reports of 76 human diazinon poisonings reported congested liver (Limaye 1966).

In laboratory animals, single oral doses of 300 mg/kg diazinon given to male and female Sprague-Dawley rats were followed by significant ($p<0.00l$-0.05) reductions in hepatic microsomal cytochrome P-450 content and in aniline hydroxylase and aminopyrine N-demethylase activities, especially during the first 24 hours. These effects largely disappeared within 72 hours to 2 weeks, with values often exceeding those of controls. No significant changes in mitochondrial respiratory function (respiratory control ratio, ADP/O ratio, and ATPase activity) were observed (Mihara et al. 1981). Oral administration of 30 mg/kg/day diazinon for 4 weeks to white male rats reduced serum betalipoprotein, alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase. Although elevated levels of these transaminases are generally associated with liver pathology, the toxicological implications of the significant reduction (13-67%) of these liver enzymes and its relevance to diazinon poisoning are unclear (Enan et al. 1982). In another rat study, normal lobular architecture was maintained in the livers, but small lipid droplets were observed in some hepatocytes after 7 weeks. In this study, male Wistar rats were treated with oral doses of 0.5 mg/kg twice a week for 28 weeks. Lipid accumulation became progressively more severe from 14 to over 28 weeks, but

no cellular necrosis was observed (at least after 14 weeks). This lipid accumulation could result from disturbed metabolism in the hepatocellular rough endoplasmic reticulum, increased lipid mobilization from peripheral tissue, or impaired lipoprotein release from liver cells. Electron microscopic examination revealed fat droplets near mitochondria, with abundant rough and smooth endoplasmic reticulum, mitochondria, and glycogen present in liver cells from both treated and control rats. No changes were observed in hepatocyte nuclei or nucleoli. But in another study, groups of rats (20 rats with 24 unexposed controls) exposed to approximately 0.18 mg/kg/day diazinon in the drinking water for 6 months exhibited no adverse effects on the liver as determined by histopathological examination (Lox and Davis 1983). The autopsy of a male Beagle dog that died from exposure to 10 mg/kg/day diazinon for an intermediate-duration (8 months) revealed fatty liver, markedly elevated serum aspartate aminotransferase, serum lactate dehydrogenase, and omithine carbamyl transferase, parenchymal atrophy, and hepatocyte dissociation (Earl et al. 1971). Female dogs treated with 20 mg/kg/day diazinon showed moderate cirrhosis, focal necrosis, fibrous infiltration, and hepatocyte dissociation. In another study, hepatic effects noted in pigs treated with 1.25 mg/kg/day for 8 months included slight inflammation and occasional lobular congestion with degenerative hepatocytes (Earl et al. 1971). Animals treated with a daily dose of 2.5 mg/kg exhibited interlobular connective tissue thickening and lobular congestion. In addition to the noted hepatic effects, all livers from swine exposed to 10 mg/kg/day were very firm to the touch and hard to cut, and one liver from a pig treated with 5 mg/kg/day diazinon was described as “friable” and very gritty, with focal subscapular hemorrhages.

An increase in relative and absolute liver weight was observed in female Sprague-Dawley rats receiving 212 mg/kg/day diazinon in feed for 13 weeks (Singh 1988). This was accompanied by histological evidence of minimal centrolobular hepatocellular hypertrophy. These findings were not seen in male rats receiving 168 mg/kg/day or females receiving 19 mg/kg/day or less. Relative liver weight was unchanged compared to controls in groups of 10 male Wistar rats receiving up to 11.7 mg/kg/day diazinon in feed for 16 weeks (Edson and Noakes 1960).

No gross or histological evidence of treatment-related damage to the liver after oral exposure to diazinon was observed in Sprague-Dawley rats receiving up to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991), in New Zealand rabbit dams receiving up to 100 mg/kg/day diazinon during gestation days 618 (Harris 1981), or in Beagle dogs (groups of 4 of each sex) receiving up to 11.6 mg/kg/day diazinon for 13 weeks (Barnes 1988).

Renal Effects. A summary of autopsy findings in 76 cases of acute diazinon poisoning described renal signs that included congested kidney and rare renal tract and kidney cortex submucosal petechiae and ecchymoses (Limaye 1966).

A single oral dose of diazinon ranging from 50 to 700 mg/kg produced dose-dependent renal effects in rats. These effects were observed to varying degrees during the first 72 hours following diazinon exposure. Substituting a purified protein diet for Purina lab chow resulted in additional oliguria, in aciduria rather than alkalinuria, and in somewhat more severe hematuria. A low-protein purified diet exacerbated the aciduria. Other renal effects included tubular swelling, capillary loop congestion, glycosuria, proteinuria, and hematuria (Boyd and Carsky 1969). Beagle dogs treated with 5 mg/kg for 8 months showed kidney corticomedullary congestion and capsular adhesions. One dog that died from exposure to 10 mg/kg/day diazinon exhibited localized chronic nephritis, tubular atrophy, and glomeruli with fibrous infiltrations (Earl et al. 1971).

Relative kidney weight was unchanged compared to controls in groups of 10 male Wistar rats receiving up to 11.7 mg/kg/day diazinon in feed for 16 weeks (Edson and Noakes 1960). No gross or histological evidence of treatment-related damage to the kidneys after oral exposure to diazinon was observed in New Zealand rabbit dams receiving up to 100 mg/kg/day diazinon during gestation days 618 (Harris 1981), in Sprague-Dawley rats (groups of 15 of each sex) receiving up to 212 mg/kg/day diazinon in feed for 13 weeks (Singh 1988), or up to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991), or in Beagle dogs (groups of 4 of each sex) receiving up to 11.6 mg/kg/day diazinon for 13 weeks (Barnes 1988).

Endocrine Effects. A 16-year-old female who drank an estimated 10 mL of a diazinon formulation (Tik-20) developed pancreatitis after being treated for cholinergic manifestations. The concentration of diazinon in the liquid was not reported so a dose could not be calculated. The pancreatic effects may well have been secondary to the diazinon-induced cholinergic manifestations (Dagli et al. 1981). Acute pancreatitis was also found in two children poisoned with diazinon (Weizman and Sofer 1992).

Pancreatic atrophy and interstitial fibrosis was reported in male Beagle dogs receiving 10 mg/kg/day diazinon in capsule form for 8 months (Earl et al. 1971), but not in females. Atrophy of the pancreatic acini was observed in male Beagle dogs receiving 10.9 mg/kg/day of diazinon in feed for

13 weeks (Barnes 1988). This effect was not observed in female Beagle dogs receiving 11.6 mg/kg/day in this study.

No gross or histological evidence of treatment-related damage to the adrenals after oral exposure to diazinon was observed in Sprague-Dawley rats (groups of 15 of each sex) receiving up to 212 mg/kg/day diazinon in feed for 13 weeks (Singh 1988). No gross or histological evidence of treatment-related damage to the adrenals, pituitary, or thyroid glands was observed in Sprague-Dawley rats receiving up to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991), or in Beagle dogs (groups of 4 of each sex) receiving up to 11.6 mg/kg/day diazinon for 13 weeks (Barnes 1988).

Ocular Effects. Miosis has been reported in humans admitted to the hospital with diazinon poisoning (Shankar 1967).

Exophthalmos has been reported in male Wistar rats receiving single doses of 50-700 mg/kg diazinon by gavage (Boyd et al. 1969; Boyd and Carsky 1969). No ocular effects were reported in Sprague-Dawley rats receiving a single dose of up to 528 mg/kg diazinon and observed for a further 14 days (Chow and Richter 1994); in Sprague-Dawley rats receiving up to 212 mg/kg/day diazinon in feed for 13 weeks (Singh 1988), or up to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991); and in Beagle dogs receiving up to 11.6 mg/kg/day diazinon in feed for 13 weeks (Barnes 1988).

Body Weight Effects. Dogs administered diazinon by the oral route in an intermediate-duration study exhibited significant weight loss at doses greater than 10 mg/kg/day. Reduced food intake, diarrhea, and emesis were also reported in this study (Earl et al. 1971). The body weight effects are probably a result of the emesis, diarrhea, generalized emaciation, and anorexia reported in the study. Significant ($p<0.05$) reductions in body weight gain were also found in male Wistar rats treated with daily oral dose of 0.5 mg/kg twice a week for 28 weeks. Body weight was significantly greater in 2%week controls (602.5 g) than in diazinon-treated rats (542.0 g) despite the absence of significant deviations in avq-age daily food intake (Anthony et al. 1986).

Significant reductions in maternal weight (5.5-9.6%) and weight gain were seen in CD-l rats receiving 100 mg/kg/day diazinon by gavage during gestation days 6-15 (Infuma et al. 1985). This effect was most striking during gestation days 6-10 when the 100 mg/kg/day group lost on average 11 grams while the control group gained 14 grams. A 25% decrease in body weight gain was seen in male

Sprague-Dawley rats receiving a single gavage dose of 264 mg/kg diazinon and observed for a period of 14 days (Chow and Richter 1994). Male Sprague-Dawley rats receiving 150.8 mg/kg/day diazinon in feed had a 15% decrease in body weight compared to controls after 6 weeks (Singh 1988). Weight gain in females was unaffected. Significantly reduced rates of body weight gain were observed in male Beagle dogs receiving 10.9 mg/kg/day diazinon in feed (34%) and in females receiving 5.6 mg/kg/day (33%) for 13 weeks (Barnes 1988). Emaciation was observed in female Beagle dogs receiving 15.99 mgikgfday diazinon in feed for 4 weeks. Less severe, but still significant, weight loss was observed in male Beagle dogs receiving 14.68 mg/kg/day (Barnes 1988).

No effects on body weight were observed in New Zealand rabbit dams receiving 100 mg/kg/day diazinon by gavage during gestation days 6-18 (Harris 1981). In studies with rats, no effect on body weight was observed in female Wistar rats receiving up to 1.35 mg/kg/day in feed for 92 days (Davies and Holub 1980a); in male Wistar rats receiving up to 11.7 mg/kg/day in feed for 16 weeks (Edson and Noakes 1960); in Wistar rats of both sexes receiving 0.21 mg/kg/day in feed for 7 days or 2.86 mg/kg/day for 30 days (Davies and Holub 1980b); and in Sprague-Dawley rats receiving up to 212 mg/kg/day in feed for 13 weeks (Singh 1988), 0.18 mg/kg/day in drinking water for 6 months (Lox and Davies 1983), or up to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991). No effects on body weight were observed in male Beagle dogs receiving 5 mg/kg/day and females receiving 10 mg/kg/day in capsules daily for 8 months (Earl et al. 1971), or in chickens receiving 2 doses of 11.3 mg/kg diazinon by gavage 21 days apart and observed for a further 21 days (Jenkins 1988).

Metabolic Effect. Metabolic acidosis was reported in patients who had ingested 240-916 mg/kg diazinon (Klemmer et al. 1978).

No effect on blood electrolytes was observed in Sprague-Dawley rats receiving up to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991).

2.2.2.3 Immunological and Lymphoreticular Effects

A summary of autopsy findings of 76 cases of acute diazinon poisoning described signs that included congested spleen (Limaye 1966).

In laboratory animal studies, single oral administration of 50-700 mg/kg diazinon to male albino Wistar rats resulted in a reduction in spleen weight (35%) and splenic red pulp contraction. Diazinon treatment also reduced thymus weight and resulted in thymic atrophy ranging from minor to near total loss of thymocytes (Boyd and Carsky 1969). One Beagle dog in the 10 mg/kg/day dose group in an intermediate-duration study in which groups of dogs were administrated oral doses of 2.5-20 mg/kg/day diazinon exhibited splenic atrophy prior to death. The spleen of an anorexic and emaciated male dog given to 10 mg/kg/day diazinon was markedly shrunken and pale in appearance with moderate atrophy in the splenic pulp prior to death after 232 days of exposure (Earl et al. 1971). The splenic atrophy reported in this study may be a result of the generalized emaciated condition of the dog due to diarrhea, emesis, and anorexia, as reported in the study.

No gross or histological evidence of treatment-related damage to the spleen or thymus after oral exposure to diazinon was observed in Sprague-Dawley rats (groups of 15 of each sex) receiving up to 212 mg/kg/day diazinon in feed for 13 weeks (Singh 1988), or up to 12 mg/kg/day for 98 weeks (Kirchner et al. 1991), or in Beagle dogs (groups of 4 of each sex) receiving up to 11.6 mg/kg/day diazinon for 13 weeks (Barnes 1988).

The highest NOAEL values and all reliable LOAEL values for immunological and lymphoreticular effects in each species and duration category are presented in Table 2-2 and plotted in Figure 2-2.

2.2.2.4 Neurological Effects

Diazinon, an anticholinesterase organophosphate, exerts its action by inhibiting neural acetylcholinesterase in the central and peripheral nervous system. Diazinon itself is a poor inhibitor of acetylcholinesterase, but is converted by mixed-function oxygenases in the liver to its oxon form, diazoxon. Diazoxon also inhibits acetylcholinesterase and is much more potent than diazinon. The extent to which this reaction takes place has a significant effect on toxicity. This inhibition results in the accumulation of -acetylcholine at acetylcholine receptors leading to muscarinic and nicotinic effects in the peripheral nervous system and central nervous system effects. These effects usually appear within a few minutes to 24 hours after dosing, depending on the extent of exposure. Signs and symptoms of diazinon-induced cholinergic effects are manifested in humans following exposure. Similarly, animals exhibit signs of diazinon cholinergic toxicity. Recovery from diazinon poisoning

results from increased availability of active acetylcholinesterase either from synthesis of new enzyme or the spontaneous hydrolysis of the enzyme-phosphate ester complex.

Acetylcholinesterase activity is also present in erythrocytes where it is known as erythrocyte acetylcholinesterase.Both forms of acetylcholinesterase are produced by the same gene and are kinetically identical (Taylor et al. 1993). In *in vitro* assays, erythrocyte and neural acetylcholinesterase are inhibited to roughly the same extent by exposure to diazinon and many other organophosphorus compounds with insecticidal activity (Iyaniwura 1991); measurement of erythrocyte acetylcholinesterase can be used as a surrogate indicator of the extent of inhibition of neural acetylcholinesterase.
A cholinesterase capable of hydrolyzing acetylcholine and butyrlcholine is produced by the liver and circulates in the blood. This enzyme, referred to as serum cholinesterase or butyrlcholinesterase, is also inhibited by diazinon and is often used as a marker for exposure. The *in vivo* substrate of this enzyme is unknown. In general, this enzyme is inhibited by diazinon at lower levels of exposure than required to inhibit neural or erythrocyte acetylcholinesterase (Barnes 1988; Singh 1988).

Signs and symptoms of cholinergic poisoning are usually seen in diazinon poisoning. In a case study of 25 persons who attempted suicide by ingesting diazinon, the most commonly reported symptom was vomiting followed by unconsciousness, giddiness, excessive sweating, and diarrhea. Clinical examinations revealed tachycardia and constricted pupils in many of the patients while some patients also became drowsy and then comatose (Kabrawala et al. 1965). A summary of autopsy findings of 76 cases of acute diazinon poisoning described signs that included congested, swollen, edematous brain with prominent dural and surface vasculature; and occasional brain hemorrhage or spinal hemorrhage (Limaye 1966). Following a suicide attempt by a woman who ingested a large quantity of diazinon, cholinergic signs were evident such as pin-point pupils and muscle fasciculation. A neurological examination showed lateral nystagmus and gross incoordination. Immediately after treatment, although the patient recovered from the acute effect, she was unable to walk or hold objects. The patient recovered withinone week (Bichile et al. 1983). A report on 5 individuals (3 male, 2 female) who intentionally ingested 60-180 mL of 25% diazinon solution (estimated to deliver a dose of 240-400 mg/kg for males and 509-986 mg/kg for females) described cholinergic signs and symptoms that included bradycardia, tachycardia, clonus, stupor, profuse diaphoresis, sialorrhea, miosis, hyperreflexia, weakness, dysdiadokinesis, abdominal pain, nausea, coma, twitching, restlessness, hyperreflexia, and bronchospasm. Many, but not all, of the listed effects were observed at one or

more time points in each of the five cases. In some cases, initial and treatment-concurrent measurements of serum and red blood cell cholinesterase activities indicated significant reduction. The victims responded well to atropine and 2-PAM treatment (Klemmer et al. 1978). Another report described an incident involving 100 persons (55 males, 45 females), most or all of whom attempted suicide by drinking diazinon formulation known as Tik-20 in India. Cases were graded according to severity of symptoms: Grade I, no clinical signs (14); Grade II, only symptoms including vomiting, diarrhea, abdominal pain, giddiness (21); Grade III, miosis with or without Grade II symptoms (27); Grade IV, pulmonary edema, with or without Grade II/III symptoms (23); and Grade V, unconsciousness, with or without Grades II-IV symptoms (15) (Balani et al. 1968). In another report of Tik-20 poisoning in India, a 16-year-old female who ingested 1.5 mg/kg diazinon (by drinking 10 mL of Tik-20) developed cholinergic signs and symptoms which included nausea, epigastric pain, headache, miosis and unreactive pupils, and tachycardia. All symptoms resolved within several hours following treatment with atropine and 2-PAM (Dagli et al. 1981). Many other studies have reported acute poisoning of persons resulting from ingestion of diazinon (Hata et al. 1986; Reichert et al. 1977; Wadia et al. 1974; Wedin et al. 1984). Other neurological effects reported in humans include petechial hemorrhages throughout the brain at autopsy in the case of a woman who ingested 293 mg/kg diazinon (Poklis et al. 1980).

In laboratory animal studies, a single oral administration of 50-700 mg/kg diazinon to albino Wistar rats resulted in signs of nervous system toxicity that included diarrhea, sialorrhea, dacryorrhea, ataxia, epistaxis, tachypnea, exophthalmos, tremors, anorexia, listlessness, diuresis, dyspnea, prostration, and hypothermia. These signs were reversible in animals which survive. Use of a low purified-protein diet did not significantly affect these signs (Boyd and Carsky 1969). Male Sprague-Dawley rats given a single oral dose of 500 mg/kg diazinon showed no abnormal clinical signs during the 48-hour period after exposure. Conversely, fasciculations, twitches, convulsions, lacrimation, chromodacryorrhea, exophthalmos, gasping, salivation, prostration, urination, and Straub tail reflex were observed in rats given a single oral dose of 1,000 mg/kg diazinon (Takahashi et al. 1991). A single 2,000 mg/kg dose resulted in substantial reductions in cholinesterase activities: in brain, 80-85%; in erythrocytes, 90%; and in serum, 50-55%. Cholinesterase activity was monitored in serum and erythrocytes of male Wistar rats 2 hours, 24 hours, 4 days, and 7 days after oral exposure to 300 mg/kg diazinon, and in the brain after 7 days (Edson and Noakes 1960). Expressed as reduction from control activity, the resulting time-course values were 42, 2, 19, and 0% (serum); 26, 89, 44, and 28% (erythrocytes); and 26% (brain). Diazinon exhibited very slow, but eventual, severe depression of red blood cell

cholinesterase activity, with only modest serum activity. Significant depression was observed at 7 days in red blood cells and brain, even though the animals had apparently clinically recovered (Edson and Noakes 1960). Albino rats were dosed once with diazinon in oil by gavage, some groups having subsequent treatment with 16 mg/kg atropine (intramuscular), with or without 30 mg/kg of pyridine 2-aldoxime methochloride given orally and/or intravenously. In rats exposed to 235 mg/kg diazinon (0.8 LD_{50}), reductions in cholinesterase activity relative to controls after 3 hours was approximately 77% in the diaphragm and 78% in the brain, and after 24 hours, 84 and 77%, respectively. By 140 hours after diazinon exposure, diaphragm activity had recovered to a 37% reduction while brain activity was still reduced by 55%. The effect of pyridine 2-aldoxime methochloride on reactivating diaphragm cholinesterase activity was examined by exposing rats to 235 mg/kg diazinon and 10 minutes later to 16 mg/kg atropine (to reduce lethality), followed 24 hours later with either oral or intravenous 30 mg/kg pyridine 2-aldoxime methochloride. One hour later, reductions in cholinesterase activity with respect to untreated controls were approximately 89% in diazinon animals, 65% in animals administered diazinon and oral pyridine 2-aldoxime methochloride, and 55% in animals administered diazinon and intravenous pyridine 2-aldoxime methochloride (Harris et al. 1969).

In an extensive study of the neurological effects of diazinon after a single oral dose (Chow and Richter 1994), groups of lo-15 Sprague-Dawley rats of both sexes were treated by gavage with doses of 2.2, 132, 264, or 528 mg/kg diazinon and observed in a functional observation battery (FOB) of tests as well as serum and erythrocyte acetylcholinesterase analysis at the expected time of peak effect (9-11 hours after dosing). FOB effects were seen only at the expected time of peak effect and not at weeks 1 or 2. Autonomic effects (with ratio affected at the lowest dose) included: altered fecal consistency (3 of 10 at 264 mg/kg), impaired respiration (6 of 10 at 528 mg/kg), lacrimation (5 of 10 at 528 mg/kg), soiled fur (3 of 10 at 264 mg/kg), stained nose (3 of 10 at 264 mg/kg), and repeated opening and closing of mouth (1 of 10 at 132 mg/kg). Neuromuscular effects noted in males included: ataxia (9 of 10 at 264 mg/kg), abnormal gait (2 of 10 at 132 mg/kg), impaired righting reflex (2 of 10 at 264 mg/kg) impaired hindlimb extensor reflex (3 of 10 at 264 mg/kg), reduced forelimb grip strength (3 of 10 at 528 mg/kg), and decreased hindlimb foot splay. Neuromuscular effects noted in females included: ataxia (3 of 10 at 132 mg/kg), abnormal gait (7 of 10 at 132 mg/kg), impaired righting reflex (2 of 10 at 264 mg/kg), impaired hindlimb extensor reflex (6 of 10 at 528 mg/kg), abnormal hindlimb positioning (3 of 10 at 528 mg/kg), and reduced forelimb and hindlimb grip strength (all at 528 mg/kg). Central nervous system activity effects consisted of decreased rearing in a

2-minute period in males at or above 264 mg/kg, and in females at or above 132 mg/kg. Central nervous system excitability effects noted in males included tremors (1 of 10 at 264 mg/kg), body twitch (5 of 10 at 264 mg/kg), lowered arousal level (all at 528 mg/kg), and easier handling (528 mg/kg). Central nervous system excitability effects noted in females included tremors (5 of 10 at 264 mg/kg), body twitch (3 of 10 at 264 mg/kg), lowered arousal level (all at 264 mg/kg), and easier handling (all at 264 mg/kg). Sensorimotor effects consisted of reduced touch response in 3 of 10 females at 528 mg/kg, and decreased tail pinch response in both sexes at 528 mg/kg. Physiologic effects consisted of reduced touch response in 3 of 10 females at 528 mg/kg, and decreased tail pinch response in both sexes at 528 mg/kg. Figure-8 maze activity was significantly suppressed (76%) in males exposed to diazinon at doses of 264 mg/kg or more ($p<0.01$). Likewise, maze activity was significantly suppressed (46%) in females exposed to diazinon at doses of 132 mg/kg or more ($p<0.01$). Doses of 528 mg/kg resulted in 97.4 and 95% decreases in maze activity among males and females, respectively. Serum cholinesterase levels were significantly reduced (28 and 53%) at the expected time of peak effect in males and females at doses >2.2 mg/kg. Erythrocyte acetylcholinesterase levels were also reduced (82%) at this time at diazinon levels >132 mg/kg in both sexes. Brain acetylcholinesterase levels, measured only at day 15, were not affected by diazinon. No gross or histopathologic changes were detected in the brain, spinal cord, peripheral nerves, skeletal muscle, eyes, or optic nerve.

Cholinergic signs of diarrhea and excessive salivation were observed in pregnant Golden Syrian hamsters orally exposed to 0.125 mg/kg/day of diazinon during gestation days 6-8 (Robens 1969). In this same study pregnant New Zealand rabbits orally exposed to 30 mg/kg/day during gestation days 6-15 exhibited these signs plus ataxia; no cholinergic signs were observed in dams exposed to 7 mg/kg/day. In another study on pregnant New Zealand rabbits orally exposed to 7, 25, or 100 mg/kg/day diazinon over gestation days 6-18 (Harris 1981), tremors and convulsions were noted in the 100 mg/kg/day group, but no cholinergic signs in the 25 mg/kg/day group.

In intermediate-duration studies, male Wistar rats were given a single oral dose of 1.75 mg/kg diazinon in 50% ethanol in water by oral gavage twice a week to study its effect on several neurotransmitters (Rajendra et al. 1986). No significant changes were noted after 7 or 14 weeks, but after 28 weeks, serum (but not erythrocyte or brain) cholinesterase activity was significantly ($p<0.05$) reduced by 49%, brain dopamine was significantly elevated by 274%, and brain gamma aminobutyric acid was reduced by 32%. The increase in brain dopamine content over control reported in this study is due to an

unexplained decline in control dopamine levels (from 0.55 µg/g at 7 weeks to 0.19 µg/g at 28 weeks). No significant changes were observed for blood serotonin, or brain aspartate, glutamate, tam-me, or glutamine (Rajendra et al. 1986). Male Wistar rats were exposed for 16 weeks to diazinon equivalent to 0.1-l1.7 mg/kg/day in their feed. At the end of the experiment, rats given 2.26 mg/kg or 11.7 mg/kg exhibited reduced serum cholinesterase activity by 17 and 52% respectively, and erythrocyte acetylcholinesterase by 46 and 79% respectively. None of these treatments produced clinical signs or changes in body weight gain, food intake, or relative liver and kidney weights (Edson and Noakes 1960). Male Wistar rats fed diazinon doses of 0 or 0.21 mg/kg/day for 7 days did not exhibit a significant reduction in serum cholinesterase or erythrocyte acetylcholinesterase activity, whereas, in female rats, serum cholinesterase activity was reduced by 29% as compared to that of untreated female controls (erythrocyte acetylcholinesterase activity was not significantly changed). These results indicate that female Wistar rats are more sensitive to diazinon toxicity than are males, and that serum cholinesterase is the more sensitive indicator of diazinon toxicity (Davies and Holub 1980b). Treatment of female Wistar rats with 0.45-l .35 mg/kg/day diazinon for 92 days, 0.09-0.36 mg/kg/day diazinon for 42 days and 0.009-0.18 mg/kg/day diazinon for 35 days in the feed also produced no visible toxic manifestations. Serum cholinesterase activity was a more sensitive indicator than erythrocyte acetylcholinesterase activity, while brain acetylcholinesterase activity was insensitive to these levels of diazinon. Maximum inhibition was achieved after 31-35 days of exposure. According to the authors, female rats were used in this study because of the greater sensitivity of females to dietary diazinon toxicity compared to males as determined in a previous study. The dose of 1.35 mg/kg/day, administered for 92 days, is considered a NOAEL for erythrocyte and brain acetylcholinesterase inhibition (Davies and Holub 1980a). In a subsequent study, Wistar rats of both sexes fed diazinon doses of 0 or 2.86 mg/kg/day for 30 days showed no clinical signs of toxicity. Diazinon treatment produced a significant depression of erythrocyte and brain acetylcholinesterase activity among treated rats at all sampling times. At all times, depression of erythrocyte acetylcholinesterase enzyme activity was greater in females than in males; at 21 days post-treatment, and continuing throughout the study, reduction of erythrocyte acetylcholinesterase activity among treated females was significantly greater (13-17%) than among treated males. Serum cholinesterase activity was reduced by 46% (males) and 69% (females) as compared to sex-paired controls by 30 days post-treatment. Erythrocyte acetylcholinesterase levels declined more gradually, reaching 30-day levels of 45% (male) and 58% (female) relative to sex-paired controls. On day 15 of treatment, brain acetylcholinesterase activity among diazinon-treated rats were not significantly different for female rats relative to sex-paired controls (5.82 µmol/g tissue/minute in controls as

compared to 5.65 μmol/g tissue/minute in diazinon-treated animals). On day 30, brain acetylcholinesterase levels were reduced by 7% for female rats relative to sex-paired controls (6.31 μmol/g tissue/minute in controls as compared to 5.89 μmol/g tissue/minute in diazinon-treated animals). It was concluded that serum cholinesterase activity is the most sensitive measure of diazinon exposure, and that female Wistar rats are more sensitive than males (Davies and Holub 1980b).

Daily oral administration of 10 or 20 mg/kg/day diazinon for 8 months resulted in fasciculations, emesis, diarrhea, with or without loss of appetite in Beagles (Earl et al. 1971). In another dog study, mixed breed female and male dogs were given diazinon in the feed at an equivalent dose of 0.006, 0.019, or 1.9 mg/kg/day for 12 weeks. While the lowest dose had no significant effect on serum cholinesterase, the intermediate dose reduced its activity to 60-80% of control and the high dose resulted in 5-30% of control activity. Enzyme activity returned to normal after 2-6 weeks. Only the highest dose (1.9 mg/kg/day) significantly reduced erythrocyte acetylcholinesterase by 55% after 12 weeks of exposure. This level was still reduced 28% 6 weeks after cessation of exposure (Williams et al. 1959). Cholinergic signs were also evident in some Hormel-Hanford pigs that were orally treated for 8 months with diazinon in capsules. Toxicity signs appeared 3-26 days after beginning the highest dose of 10 mg/kg/day (Earl et al. 1971).

Neurological effects observed in Sprague-Dawley rats receiving 0.04, 0.2, 8.4, or 150.8 mg/kg/day diazinon in feed (males) and 0.05, 0.2, 9.4, or 183.2 (females) in feed for 6 weeks (Singh 1988) included statistically significant decreases in erythrocyte acetylcholinesterase at 24 days in males at 8.4 mg/kg/day (21%) and at 150.8 mglkgtday (20%), and at 24 days in females at 9.4 mg/kg/day (21%) and 183.2 mg/kg/day (25%). Statistically significant decreases in brain acetylcholinesterase were seen in males at the termination of the study at 150.8 mg/kg/day (58%), and in females at 9.4 mg/kg/day (24%) and at 183.2 mg/kg/day (61%). No treatment-related effects were noted for absolute or relative brain weights. A 13-week study using similar doses (Singh 1988) reported treatment-related clinical symptoms including hypersensitivity to touch and sound in the 168 mg/kg males and 212 rng/kg females. Males in the 168 mg/kg dose group also exhibited aggressive behavior. At termination, hyperactivity was noted in the 168 mg/kg males and 212 mg/kg females. Statistically significant decreases were seen in erythrocyte acetylcholinesterase in males at 15.0 mg/kg/day (27%) and at 168 mg/kg/day (26%) and in females at 0.4 mg/kg/day (17%), 19 mg/kg/day (41%) and at 212 mg/kg/day (41%). Statistically significant decreases were seen in brain acetylcholinesterase in males at 168 mg/kg/day (49%), and in females at 19 mg/kg/day (41%) and 212 mg/kg/day (57%). No

gross or histopathologic changes were detected in the brain, spinal cord, peripheral nerves, skeletal muscle, eyes, or optic nerve.

Beagle dogs receiving doses of 0.02, 0.073, 0.8, or 14.68 mg/kg/day diazinon in males and 0.023, 0.082, 0.75, or 15.99 mg/kg/day (females) in feed for 4 weeks (Barnes 1988) exhibited statistically significant decreases in erythrocyte acetylcholinesterase in males at 0.073 mg/kg/day (11%) and at 14.68 mg/kg/day (30%), and in females at 15.99 mg/kg/day (38%). Statistically significant decreases in brain acetylcholinesterase were seen in males at 14.68 mg/kg/day (44%) and in females at 15.99 mg/kg/day (50%). No treatment-related changes in absolute or relative brain weights were noted. In a 13-week study using similar doses (Barnes 1988) statistically significant reductions in erythrocyte and brain acetylcholinesterase levels were noted in males and females beginning at the 5.9 and 5.6 mg/kg levels respectively. No change was observed in blood drawn on day 12. On days 29, 56, and 86 erythrocyte acetylcholinesterase declined by 26, 25, and 25% in males and 31, 31, and 31% in females at these doses. Levels in the highest dose groups (10.9 and 11.6 mg/kg/day) were similar. Brain samples analyzed at the termination of the study showed reduction of acetylcholinesterase activity of 31% in males at 5.9 mg/kg/day and 42% at 10.9 mg/kg/day. Female brain acetylcholinesterase activity was reduced 30% at 5.6 mg/kg/day and 45% at 11.6 mg/kg/day. Clinical signs included emesis and diarrhea, but were not dose related. No pathology of any nervous tissues were noted under either gross or microscopic examination.

In a chronic-duration study in Sprague-Dawley rats, diazinon was administered in feed at doses of 0.004, 0.06, 5, or 10 mg/kg/day (males) and 0.005, 0.07, 6, or 12 mg/kg/day (females) for 52 or 98 weeks (Kirchner 1991). Clinical signs of organophosphate neurological toxicity (diarrhea, salivation, lacrimation, tremor, etc.) were not observed in any treated groups. After one year, erythrocyte acetylcholinesterase was decreased 16 and 11% at 5 and 10 mg/kg/day, respectively, in males and 22 and 20% at 6 and 12 mg/kg/day in females, respectively. During a subsequent 4-week recovery period, male erythrocyte acetylcholinesterase returned to normal, while that of females dosed at 12 mg/kg/day-was still decreased by 7%. Results were similar at 98 weeks: decreases of 21 and 22% in males at 5 and 10 mg/kg/day, respectively, and decreases in females of 26 and 25% at 6 and 12 mg/kg/day, respectively. No statistically significant reduction in brain acetylcholinesterase was observed in males after 1 year; however, activity in females decreased by 26 and 40% at doses of 6 and 12 mg/kg/day, respectively. During a 4-week recovery period, brain acetylcholinesterase activity in the 12 mg/kg/day female group recovered from 40% to 9% inhibition. After 98 weeks, significant

decreases in brain acetylcholinesterase activity were present in males. Decreases of 24 and 42% were observed in the 5 and 10 mg/kg/day groups, respectively. Female brain acetylcholinesterase inhibition at 98 weeks was similar to that seen after 1 year. Decreases of 29 and 48% were observed in the 6 and 12 mg/kg/day groups, respectively. No histopathologic evidence of treatment-related damage was observed in the brain, optic nerves, spinal cord, or sciatic nerve, at either 52 or 98 weeks.

Diazinon MG-8 (purity 87%) has been tested for organophosphate-induced delayed neurotoxicity in chickens (Jenkins 1988). Ten hens (Red Heavy breed) were used as a control group and received 1.0 mL/kg of corn oil by gavage on days 0 and 21 of the study. Eighteen hens received 11.3 mg/kg diazinon in 1.0 mL of corn oil by gavage on days 0 and 21 (the approximate LD_{50} in hens for this chemical). A positive control group of 8 hens received 500 mg/kg of the known delayed neurotoxicant tri-0-tolyl phosphate. Pretreatment with atropine (10 mg/kg) one hour before dosing and treatment at dosing with 2-PAM (50 mg/kg) were used to protect against acute cholinergic effects in all groups. All hens were also treated with atropine and 2-PAM one and five hours after dosing. Two hens required and received protective treatment the following day. All hens were observed and scored independently for signs of delayed neurotoxicity (gait disturbances, ataxia) by two different observers three times weekly. By day 13, all the positive control hens showed some degree of neurotoxicity (unsteadiness in walking to marked staggering and falling). None of the diazinon-treated hens displayed these signs. These hens were treated again on day 21 with diazinon (11.3 mg/kg) and observed for a further 3 weeks. One hen exhibited a slight unsteadiness in walking on day 41. On day 43, the hens were sacrificed and brain, spinal cord, and peripheral nerve from control, positive control, and treated hens were prepared for histopathology. Histopathological examination revealed no lesions consistent with delayed neurotoxicity in the control or treated hens, although these lesions were seen in the positive control hens.

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category are presented in Table 2-2 and plotted in Figures 2-2.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to diazinon. Limited information from laboratory experimentation indicated that, although oral diazinon exposure did not produce any significant effects on reproduction in four generations of rats, testicular atrophy

and arrested spermatogenesis were seen in dogs. In one study, no adverse effects on reproduction were reported for mature female Sprague-Dawley rats fed 0.05 mg/kg/day diazinon in the diet for 60 days prior to weaning for 4 generations. No adverse effect on fertility was observed, as all females became pregnant. Apparently, diazinon exposure increased the average number of pups per litter compared to undosed controls (9.7-l 1.1 as opposed to 6.2-S) for all 5 generations (F_0-F_4) of offspring (Green 1970). In another study in hybrid mice, litter size was reduced by 20% at oral maternal diazinon doses of 0.18, but not at 9 mg/kg/day relative to controls (Spyker and Avery 1977). A 14% reduction in maternal weight gain was observed at both doses. Male and female Beagle dogs were given daily capsules containing diazinon in corn oil in doses ranging from 2.5 to 20 mg/kg/day for 8 months. Testicular atrophy with completely arrested spermatogenesis was observed only in the one male dog of the 10 mg/kg/day group that lost weight and evidenced other gross pathological changes. All 3 male dogs in the 20 mg/kg/day group suffered similar effects (testicular atrophy observed in 2 of 3, arrested spermatogenesis observed in 1 of 3) (Earl et al. 1971).

Administration of diazinon at 10, 20, or 100 mg/kg/day diazinon by oral gavage during gestation days 6-15 in CD-l rats had no significant effect on litter sizes or numbers of viable fetuses (Infuma et al. 1985). Maternal toxicity was noted at 100 mg/kg/day with a significant reduction in feed consumption and body weight gain. In New Zealand rabbits dosed by gavage at 7, 25, or 100 mg/kg/day diazinon during gestation days 6-l 8, no differences with controls were seen in number of implantations, proportion of live, dead or resorbed fetuses, fetal weights, or fetal sex ratios (Harris 1981). Nine of 22 does treated at 100 mg/kg/day died during the course of the study so significant maternal toxicity at this dose was observed.

No gross or histological evidence of treatment-related damage to reproductive tissues (ovaries, uterus, vagina, epididymides, seminal vesicles, testes) was observed in Sprague-Dawley rats (groups of 15 of each sex) exposed to up to 168 mg/kg/day diazinon (males) or 212 mg/kg/day (females) for 13 weeks via feed (Singh 1988), or exposed up to 10 mg/kg/day (males), or 12 mg/kg/day (females) for 98 weeks (Kirchner et al. 1991), or in Beagle dogs (groups of 4 of each sex) exposed to-up to 10.9 mg/kg/day (males) or 11.6 mg/kg/day (females) (Barnes 1988).

The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category are presented in Table 2-2 and plotted in Figure 2-2.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects of diazinon in humans after oral exposure. Laboratory animal studies with mice provide evidence that exposure to diazinon via mother's milk does not result in neonatal toxicity. Results of toxicity evaluation in rats, mice, hamsters, and rabbits also indicate that oral exposure to diazinon does not have dose-response effects on the developing mammalian fetus or neonate. The adverse effects reported for pups have been suggested to derive from diazinon impairment of placental transport of nutrients or maternal regulation of fetal growth, or directly via antagonism to cholinergic development of the fetus. No significant effects were seen in rabbit offspring at maternally lethal doses.

Pregnant Wistar rats given single doses of 52.6 or 63.5 mg/kg on gestation day 10 resulted in no deaths, and multiple doses of 6.6-17.3 mg/kg/day given on gestation day 8-12 or 12-15 resulted in only one maternal death. Little effect was observed on average fetal weight. Implantation per dam was not affected, but higher doses (70.6, 89.9, 95.2 mg/kg) increased fetal resorptions; this effect was seen only at the lowest dose tested (68.3 mg/kg) in rats treated only on gestation day 9. Both visceral (hydroureter, hydronephrosis) and skeletal (rudimentary/short/wavy ribs, irregular bone contours, hypocalcification) abnormalities were observed only at the lowest dose tested (68.3 mg/kg) (Dobbins 1967).

In a teratology study, a mouse dihybrid F_2, strain, obtained by crossing F_1 (female C57BL/6 x male A/JAX) with F_1 hybrid HC (female C3WHe x male Balb/c) was used as a more vigorous strain that was well-buffered against spontaneous congenital deformities. Dams were exposed to doses of 0, 0.18, or 9 mg/kg/day diazinon in peanut butter throughout gestation. The study found no maternal toxicity at any of the doses tested. However, the study reported significantly elevated ($p<0.05$) mortality (12%, 18 of 150) in the high dose group at weaning (postpartum day ZS), but not in the low dose group (2%, 3 of 134), when compared with controls (6%, 19 of 311). Histological examination indicated that the majority of these pups died from pulmonary congestion and mucosal infiltration consistent with acute bronchitis. Diazinon treatment did not adversely affect mortality from after weaning. Exposure to diazinon via mother's milk did not have any adverse effect (Bamett et al. 1980). A previous study using the same protocol and dose regimen exposed dams throughout gestation to doses of 0, 0.18, or 9 mg/kg/day diazinon in peanut butter (Spyker and Avery 1977). Dams exposed to either diazinon dose experienced reduced weight gain (86% that of controls, $p<0.05$) during

pregnancy, but gestation length was not significantly affected. Pup weight gain during the first 14 weeks after parturition was significantly ($p<0.05$) less at the 9 mg/kg/day dose than at the 0 and 0.18 mg/kg/day doses. With the exception of contact placing and sexual maturity, which were delayed with respect to controls ($p<0.05$) in the low dose pups, developmental ontogeny as measured by numerous parameters was not significantly affected by diazinon exposure. No teratological effects were evident. However, both diazinon groups displayed endurance and coordination deficits during neuromuscular function tests (rod cling and inclined plane), and 9 mg/kg/day offspring also displayed slower running speed in a Lashley II maze and reduced swimming endurance. Morphologically, the brains of 9 (but not 0.18) mg/kg/day offspring had focal abnormalities in the forebrain area, including dense aggregations of atypical chromatin-containing cells. Among the offspring of hybrid mice dams exposed to 0.18 or 9 mg/kg/day diazinon during gestation days l-l 8, females from the 9 mg/kg/day group showed a 33% decrease of serum IgG_l levels 101 days after birth (Barnett et al. 1980). These levels were normal at 400 and 800 days after birth, no effects on serum Ig levels were observed in male offspring at either dose. Fetal exposure to low levels of diazinon may result in functional deficits in otherwise normal animals that can only be detected by systemic behavioral evaluation. These neural dysfunctions and pathologies might occur either indirectly through diazinon impairment of placental transport of nutrients or maternal regulation of fetal growth, or directly via antagonism to cholinergic development of the fetus (Spyker and Avery 1977).

Pregnant Golden Syrian hamsters were orally exposed by gavage to diazinon during organogenesis (0.125 mg/kg/day to 8 dams on gestation day 6, 7, and 8; 0.25 mg/kg/day to 5 dams on gestation day 7 or 8). All dams evidenced cholinergic signs of diarrhea, salivation, ataxia, but no deaths. No terata were observed at either dose, nor were average number of fetuses per litter, fetal mortality, or average fetal weight adversely affected. Thus, at maternally toxic doses, diazinon was not feto- or developmentally toxic to hamsters (Robens 1969).

Diazinon was not fetotoxic or developmentally toxic to rabbits in maternally lethal doses. When pregnant New Zealand white rabbits were orally exposed by gel capsules to 7 or 30 mg/kg/day diazinon on gestation day 15, 6 of 8 of the dams in the high dose group died. The dams in this dose group also exhibited severe cholinergic signs. However, no terata or dose-related embryotoxic (average number of fetuses per litter, fetal mortality, average fetal weight) effects were observed even at maternally toxic doses (Robens 1969). In another study where New Zealand rabbit does were exposed by gavage to 7, 25, or 100 mg/kg/day diazinon during gestation days 6-18 and sacrificed on

gestation day 25, no significant treatment-related fetal malformations or skeletal malformations were observed in the offspring (Harris 1981). Nine of the 22 does in the 100 mg/kg/day group died during the study, indicating that significant maternal toxicity took place at this dose.

An increased incidence of rudimentary ribs at T-14 was observed in CD-l rats receiving 100 mg/kg/day diazinon during gestation days 6-15 (Infuma et al. 1985). This finding was accompanied by severe weight loss in the dams and this developmental effect was considered by the authors of this study to be secondary to maternal toxicity.

The highest NOAEL values and all reliable LOAEL values for developmental effects in each species and duration category are presented in Table 2-2 and plotted in Figure 2-2.

2.2.2.7 Genotoxic Effects

Chronic occupational exposure to multiple insecticides, including diazinon, has been associated with increased incidence of chromosomal aberrations and increased sister chromatid exchanges in peripheral blood lymphocytes compared with non-exposed populations (de Ferrari et al. 1991; Kiraly et al. 1979; See et al. 1990). Some of these exposures are presumed to be oral. However, it is not possible to attribute the results of these studies to diazinon alone as workers were exposed to up to 80 different insecticides in unknown amounts for variable durations.

No studies were located regarding genotoxic effects in animals after oral exposure to diazinon. Other genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

Several epidemiological studies have reported increased incidence of cancers in humans who were concurrently or sequentially exposed to a number of insecticides, including diazinon. However, it is not possible to attribute the increased cancer incidence exclusively to diazinon exposure. Some of the exposure is presumed to have occurred by the oral route.

A case-control study suggested a possible link between family gardening use of diazinon (and other insecticides) and increased incidence of childhood brain cancer (type unspecified). However, this

report gave no indication of level, duration, or frequency of exposure to diazinon (or to other insecticides) (Davis et al. 1993). Another case-control study suggested a positive association between an increased incidence of non-Hodgkin's lymphoma in farmers compared to non-farmers. The report attributed the increased incidence of lymphomas to handling of organophosphorus insecticides, including diazinon (Cantor et al. 1992). A third case-control study suggested an association between an increased incidence of multiple myelomas and high exposure to insecticides, including diazinon. Actual exposure to diazinon was reported in 2 (0.3%) of the cases and 5 (0.3%) of the controls (Morris et al. 1986).

In laboratory animal studies, a cancer bioassay was conducted with groups of 100 (50 male, 50 female) Fischer 344 rats in which the rats were exposed *ad libitum* to an estimated 0, 20, or 40 mg/kg/day dose of diazinon in their feed for 103 weeks. Rats (25 of each sex) were used as controls. Tissue masses were noted especially in high-dose males and low-dose females, and tachypnea incidence was elevated in exposed groups. A variety of neoplastic and non-neoplastic lesions were observed with approximately equal frequency in the control and dosed groups in both sexes. An increase in the common lesion of endometrial stromal polyps was observed in female rats (control = 2 of 23, low dose = 8 of 43, high dose = 11 of 49) was considered unrelated to diazinon exposure. In male rats, lymphomas and leukemias were significantly ($p<0.011$) elevated in the low dose group (25 of 50), but not in the high dose group (12 of 50), relative to controls (5 of 25). The study concluded that diazinon was not carcinogenic under the conditions of this assay for either sex of Fischer 344 rats (NCI 1979). In another cancer bioassay, groups of 100 (50 of each sex) $B6C3F_1$ mice were exposed for 103 weeks to dietary concentrations of 0, 13, or 26 mg/kg/day. Fifty mice (25 of each sex) were used as controls. A number of neoplastic and non-neoplastic lesions, essentially considered non-treatment-related, were observed in both the control and treated mice. An elevation in hepatocellular adenomas and carcinomas was observed in low-dose mice (20 of 46), but not in the high-dose mice (13 of 48), relative to controls (5 of 21). The study concluded that diazinon was not carcinogenic under the conditions of this assay for either sex of $B6C3F_1$ mice (NCI 1979).

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to diazinon.

In laboratory animal studies, the acute dermal toxicity of diazinon and its formulations varies profoundly, largely as the result of sample aging and differences in purity of formulation and solvents used. In general, aged diazinon samples that contained more impurities were more toxic (Gaines 1960). The use of an occlusive dressing after dermal application usually increases dermal toxicity because it enhances sweating and dermal absorption. Dermal LD_{50} values were determined in Sherman rats of both sexes (Gaines 1960). Diazinon was applied to a shaved dermal area of approximately 13.5 cm^2. LD_{50} values were 900 mg/kg and 455 mg/kg for males and females, respectively. The dermal LD_{50} of emulsifiable solution of diazinon was determined in male Wistar rats (Noakes and Sanderson 1969). When applied doses were occluded by a plaster cover backed with aluminum foil, LD50 values were 1,100 mg/kg and 500 mg/kg after 4 and 24 hours, respectively. In another study, either emulsified solution or wettable powder formulations of diazinon were applied on the shaved skin of male Wistar rats. Applications were covered with a “plastic girdle” which was removed after 20 hours when the animals were thoroughly decontaminated by washing. No deaths or clinical signs were observed during the 7-day observation period after a maximum dose of 1000 mg/kg (Edson and Noakes 1960). Among New Zealand rabbits dermally exposed for 24 hours at 2,020 mg/kg to the diazinon MG-8 formulation (purity not reported but probably about 87%), 2 of 5 females died 2 days after exposure ceased (Kuhn 1989b). None of five males died.

A total of 25 male and 91 female sheep was dipped (5 minutes for adults and 3 minutes for lambs) in a 0.02% emulsion of diazinon (Smith 1970). One of 23 ewes and 1 of 3 ewe lambs died after exhibiting salivation, fasciculation, dyspnea, lethargy, ataxia, and miosis. Whole blood cholinesterase activity was reduced.

The LD_{50} values and doses associated with death in each species and duration category are shown in Table 2-3.

2.2.3.2 Systemic Effects

No studies were located regarding musculoskeletal, renal, ocular, or body weight effects in humans after dermal exposure to diazinon. No studies were located regarding cardiovascular, hematological, musculoskeletal, renal, endocrine, or ocular effects in animals after dermal exposure to diazinon.

Table 2-3. Levels of Significant Exposure to Diazinon - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference
ACUTE EXPOSURE						
Death						
Rat (Sherman)	once				900 M mg/kg (LD_{50}) 455 F mg/kg (LD_{50})	Gaines 1960
Rat (Wistar)	4 or 24 hr				1100 M mg/kg (estimated LD_{50}, 4-hour) 500 M mg/kg (LD_{50}, 24-hour)	Noakes and Sanderson 1969
Systemic						
Human	72 hr	Dermal	1%			Lisi et al. 1987
Gn Pig (Hartley)	24 hr	Dermal	5% F	10% F (erythema)		Matsushita et al. 1985
Rabbit (New Zealand)	4 hr	Dermal		0.5 mL (erythema, slight edema)		Kuhn 1989a
Immunological/Lymphoreticular						
Human	once		1%			Lisi et al. 1987
INTERMEDIATE EXPOSURE						
Systemic						
Rat (dark agouti)	12 wk 7 d/wk 1 x/d	Hepatic		114 F mg/kg (elevated fecal porphyrin)		Bleakeley et al. 1979

Table 2-3. Levels of Significant Exposure to Diazinon - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL		Reference
				Less Serious	Serious	
Immunological/Lymphbreticular						
Gn Pig (Hartley)	24 hr			0.05% F (moderate delayed contact sensitivity)		Matsushita et al. 1985

d = day(s); F = female; Gn pig = Guinea pig; hr = hour(s); LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observable-adverse-effect level; M = male; NOAEL = no-observable-adverse-effect level; NS = not specified; wk = week(s); x = times

Respiratory Effects. A 56-year-old female gardener, dermally exposed to spilled diazinon of unknown purity, developed respiratory distress as a component of the spectrum of the symptoms of cholinergic effects resulting from acetylcholinesterase inhibition. The victim exhibited pulmonary edema with bilateral lung crepitations and tachypnea (Lee 1989).

Nasal discharge was observed in New Zealand rabbits of both sexes following dermal exposure for 24 hours to 2,020 mg/kg of the diazinon MG-8 formulation (Kuhn 1989b). The purity of this formulation was not reported but was probably about 87% based on similar reports for the MG-8 formulation reported by the manufacturer, Ciba-Geigy.

Cardiovascular Effects. A 56-year-old female gardener, dermally exposed to spilled diazinon of unknown purity, developed sinus tachycardia with no evidence of infarction and showed increased cardiac enzyme (serum glutamate oxalate transaminase, total lactate dehydrogenase creatine phosphokinase) levels. The victim was diagnosed on discharge with acute left ventricular failure (Lee 1989).

Gastrointestinal Effects. In a case report, two female gardeners, dermally exposed to spilled diazinon of unknown purity, developed signs of acute pancreatitis which included abdominal colic, diarrhea, nausea, vomiting, and epigastric pain, as well as elevated serum amylase and urinary diastase levels (Lee 1989).

Both decreased defecation and diarrhea were observed in New Zealand rabbits of both sexes following dermal exposure for 24 hours to 2,020 mg/kg of the diazinon MG-8 formulation (Kuhn 1989b). The purity of this formulation was not reported but was probably about 87% based on similar reports for the MG-8 formulation reported by the manufacturer, Ciba-Geigy.

Hematological Effects. Two female gardeners, dermally exposed to spilled diazinon of unknown purity, developed hypokalemia and leucocytosis (Lee 1989).

Hepatic Effects. Female dark Agouti rats received daily cutaneous doses of either 114 or 229 mg/kg/day diazinon (as 85% technical grade solution). Significant elevations in total fecal porphyrin excretion were observed at the 114 mg/mg/day dose after 8-12 weeks (3-5-fold), and at the 229 mg/kg/day dose at least by week 12 (4-fold). No concomitant rises in urinary porphyrin excretion

were observed. Electrophoretic analysis revealed the presence of isocoporphyrin in the feces. Except for the unexplained lack of urinary porphyrin, these findings were noted to be biochemically characteristic of human porphyria cutanea tarda, and indicative of disturbed hepatic porphyrin metabolism. However, oral administration of 46 mg/kg/day to another group of rats was without this effect (Bleakley et al. 1979).

Endocrine Effects. Two female gardeners, 56 and 48 years old, dermally exposed to spilled diazinon of unknown purity, developed signs of acute pancreatitis which included abdominal colic, diarrhea, nausea, vomiting, and epigastric pain, as well as elevated serum amylase and urinary diastase levels. One of the victims was diagnosed on discharge with organophosphate poisoning and diabetes mellitus. The authors of this study noted that acute pancreatitis is frequently a component of organophosphate intoxication, although it is often not recognized as such in the medical literature or by treating physicians (Lee 1989).

Dermal Effects. Dermal exposure to diazinon resulted in contact dermatitis in farm workers (Matsushita et al. 1985). But, according to another report, a 1% diazinon solution in a skin patch did not elicit an irritation or cause sensitization in humans (Lisi et al. 1987).

Skin erythema was noted in guinea pigs dermally exposed to 10 and 20% diazinon, but not at lower concentrations of 0.5-5.0%) (Matsushita et al. 1985). The purity of the diazinon used was not reported in this study. Well defined erythema and slight edema were observed in New Zealand rabbits of both sexes following dermal exposure for 4 hours to 0.5 mL of the diazinon MG-8 formulation (Kuhn 1989a). The purity of this formulation was not reported, but was probably about 87% based on similar reports for the MG-8 formulation reported by the manufacturer, Ciba-Geigy.

Body Weight Effects. Body weight was unaffected in New Zealand rabbits dermally exposed to up to 2,020 mg/kg for 24 hours to the diazinon MG-8 formulation (purity not reported but probably about 87%) and observed for a further 14 days (Kuhn 1989b).

2.2.3.3 Immunological and Lymphoreticular Effects

One percent diazinon in “pet.” (presumably petroleum ether) has been tested for allergic reactions by patch tests in 294 volunteers examined after 48 and 72 hours of dermal contact (Lisi et al. 1987). The 1% diazinon solution on a skin patch did not elicit allergic reactions in any of the volunteers studied.

Diazinon has also been tested for delayed contact hypersensitivity following skin application to guinea pigs. Induction concentrations of diazinon were reported as 5% (intradermal) and 25% (topical). At both 24 and 48 hours after challenge in the guinea pig maximization test, response to a 0.05% diazinon challenge was scored as grade III (moderate, 30% sensitization rate), and to 0.5% diazinon as grade V (extreme, 100% rate). When cross-sensitization was tested using a challenge of 0.2 or 2% benomyl, allergenicities were grade I (0%) and grade III (30%), respectively (Matsushita et al. 1985). Skin sensitization did not occur in Hartley guinea pigs treated 11 times over a 36-day period with 0.5 mL of the diazinon formulation MG-8 (purity not reported but probably about 87%) (Kuhn 1989~).

2.2.3.4 Neurological Effects

In a case report, two female gardeners, 56 and 48 years old, dermally exposed to spilled diazinon of unknown purity, developed cholinergic organophosphate poisoning symptoms. The victims exhibited signs and symptoms which included cyanosis, frothing at the mouth, drowsiness, nausea, vomiting, abdominal colic, diarrhea, tachypnea, miosis, and sinus tachycardia with no evidence of infarction. One victim showed significantly depressed serum cholinesterase levels (Lee 1989).

Tremors were reported in female New Zealand rabbits but not males after 24 hours of dermal exposure to 2,020 mg/kg of the diazinon formulation MG-8 (purity not reported but probably about 87%) (Kuhn 1989a).

No studies were-located regarding organophosphate-induced delayed neurotoxicity (OPIDN) in humans or in animals after dermal exposure to diazinon.

2.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following dermal exposure to diazinon.

Within 36-48 hours after the dipping of 25 male and 91 female sheep, premature (by 3 months) ovulation and estrus were observed in 5 of 23 ewes (Smith 1970). Toxic effects were limited only to the Dorset Down breed, which suggests a partial species-specific response.

2.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after dermal exposure to diazinon.

2.2.3.7 Genotoxic Effects

Chronic-duration occupational exposure to multiple insecticides, including diazinon, has been associated with increased incidence of chromosomal aberrations and increased sister chromatid exchanges in peripheral blood lymphocytes compared with non-exposed populations (de Ferrari et al. 1991; Kiraly et al. 1979; See et al. 1990). Some of these exposures are presumed to be through the skin. However, it is not possible to attribute the results of these studies to diazinon alone as workers were exposed to up to 80 different insecticides in unknown amounts for variable durations. No studies were located regarding genotoxic effects in animals after dermal exposure to diazinon. Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

Several epidemiological studies have reported increased incidence of cancers in humans who were concurrently or sequentially exposed to a number of insecticides, including diazinon. However, it is not possible to attribute the increased cancer incidence exclusively to diazinon exposure. Some of the exposure is presumed to have occurred by dermal exposure.

A case-control study suggested a possible link between family gardening use of diazinon (and other insecticides) and increased incidence of childhood brain cancer (type unspecified). However, this report gave no indication of level, duration, or frequency of exposure to diazinon, or to other insecticides (Davis et al. 1993). Another case-control study suggested a positive association between an increased incidence of non-Hodgkin's lymphoma in farmers compared to non-farmers. The report attributed the increased incidence of lymphomas to handling of organophosphorus insecticides, including diazinon (Cantor et al. 1992). A third case-control study suggested an association between an increased incidence of multiple myelomas and high exposure to insecticides, including diazinon. Actual exposure to diazinon was reported in 2 (0.3%) of the cases and 5 (0.3%) of the controls (Morris et al. 1986).

No studies were located regarding cancer in animals after dermal exposure to diazinon.

2. 3 TOXICOKINETICS

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

No studies were located regarding absorption after inhalation exposure of diazinon in humans or animals.

2.3.1.2 Oral Exposure

Diazinon was detected in several tissues from a woman who had ingested a lethal amount of an estimated 293 mg/kg diazinon formulation ("FERTI-LOME" bagworm spray) containing 10% diazinon suggesting rapid absorption from the gastrointestinal tract (Poklis et al. 1980). Animal studies also confirmed the rapid absorption of diazinon following oral administration of [^{14}C]diazinon. Wistar WU rats of both sexes were given either a single oral dose of 4 mg/kg or daily doses of 8.0 mg/kg for 10 consecutive days of [^{14}C]diazinon. The rapid absorption of diazinon was indicated by the early excretion of radioactivity (Mücke et al. 1970). Similar results were obtained following a single oral dose of 4.0 mg/kg [^{14}C]diazinon to female Beagle dogs where absorption was determined to be at least 85% (Iverson et al. 1975). In goats given daily oral doses of 0.5 or 5.0 mg/kg/day diazinon for

7 days or a single 150 mg/kg or 700 mg/kg dose, diazinon was detected from the first day of treatment (Mount 1984). Other studies demonstrated rapid absorption of orally administered diazinon in sheep (Janes et al. 1973; Machin et al. 1971, 1974) and in cows (Abdelsalam and Ford 1986).

2.3.1.3 Dermal Exposure

Volunteers were exposed for 24 hours to [^{14}C]diazinon applied to either the forearm or abdomen in acetone or lanolin wool grease (Wester et al. 1993). Absorption was determined to be 34% of the applied dose with no difference related to vehicle or the area applied.

No studies were located regarding absorption of diazinon after dermal exposure in animals.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding distribution of diazinon after inhalation exposure in humans or animals.

2.3.2.2 Oral Exposure

Samples of stomach contents, blood, bile, adipose tissue, liver, brain, and kidney were collected at autopsy of a woman who ingested a lethal dose estimated at 293 mg/kg of a diazinon formulation ("FERTI-LOME" bagworm spray) containing 10% diazinon (Poklis et al. 1980). The highest concentrations were found in the blood, followed by stomach contents and the bile. Lowest concentrations were found in the kidney, followed by adipose tissue, and then bile. Animal studies confirmed the human case study in that diazinon is widely distributed in all analyzed tissues in rats (Mticke et al. 1970), in sheep (Janes et al. 1973; Machin et al. 1971, 1974), and in cows (Abdelsalam and Ford 1986).

2.3.2.3 Dermal Exposure

No studies were located regarding distribution of diazinon after dermal exposure in humans or animals.

2.3.2.4 Other Exposure

Two female Beagle dogs received a single intravenous dose of 0.2 mg/kg [^{14}C]diazinon in ethanol. Radioactivity in the blood decreased in a biphasic manner. The half-life of the terminal or elimination phase was estimated to be 1.5 hours (Iverson et al. 1975). Diazinon was found (5 mg/kg) in omental fat of a man found unconscious and who died 11 days later (Kirkbride 1987). Pesticide exposure was suspected in his death but no confirmatory test of acetylcholinesterase activity was performed, nor were clinical signs of diazinon toxicity reported. This man worked at a horticultural supply store and was an active gardener, however no route of exposure to the diazinon could be confirmed.

2.3.3 Metabolism

2.3.3.1 Inhalation Exposure

Diethylthiophosphate (DETP), a potential diazinon metabolite, was found in urinary samples from pest control operators exposed to diazinon by inhalation (Weisskopf et al. 1988).

2.3.3.2 Oral Exposure

Diazinon was the only chemical detected in the body fluids and tissues from a woman who died after ingesting a lethal dose estimated at 293 mg/kg of a diazinon formulation (“FERTI-LOME” bagworm spray) containing 10% diazinon (Poklis et al. 1980). A report on 5 individuals (3 males, 2 females) who intentionally ingested 60-180 mL of 25% diazinon solution (estimated to deliver a dose of 240-400 mg/kg for males and 509-986 mg/kg for females) found diazinon in serum and several metabolites (monoethyl phosphate, diethyl phosphate, diethyl phosphorothioate) in the urine (Klemmer et al. 1978). In rats given a single oral dose of unlabeled diazinon and ring-labeled [^{14}C]diazinon, complete degradation of the pyrimidine ring to $^{14}C0_2$ did not take place. Hydrolysis of the ester bond yielding 2-isopropyl-4-methyl-6-hydroxypyrimidine and diethyl phosphate (diazoxon) or diethyl phosphothiorate (diazinon) was determined to be the main degradative pathway of labeled diazinon (Machin et al. 1975; Mücke et al. 1970).

2.3.3.3 Dermal Exposure

No studies were located regarding metabolism of diazinon after dermal exposure in humans or animals.

2.3.3.4 Other Exposure

Diethyl phosphorothioic acid and diethyl phosphoric acid have been identified as metabolites of [^{14}C]diazinon following a single intravenous injection in female Beagle dogs (Iverson et al. 1975). In vitro metabolism of ethoxy-l-[^{14}C]diazinon was carried out using rat liver microsomes (Yang et al. 1971). Diazinon was metabolized in a complex reaction via cytochrome P-450 to diazoxon and 2-isopropyl-4-methyl-6-hydroxypyrimidine. Diazoxon hydrolysis was catalyzed by microsomal enzymes without requiring NADPH to yield diethyl phosphoric acid. Diazoxon did not undergo desethylation reaction. Similar results were obtained using liver microsomes from sheep, cow, pig, rat, turkey, chicken, and duck (Machin et al. 1975).

The proposed metabolic pathway of diazinon (Aizawa 1989; Yang et al. 1971) is shown in Figure 2-3.

2.3.4 Excretion

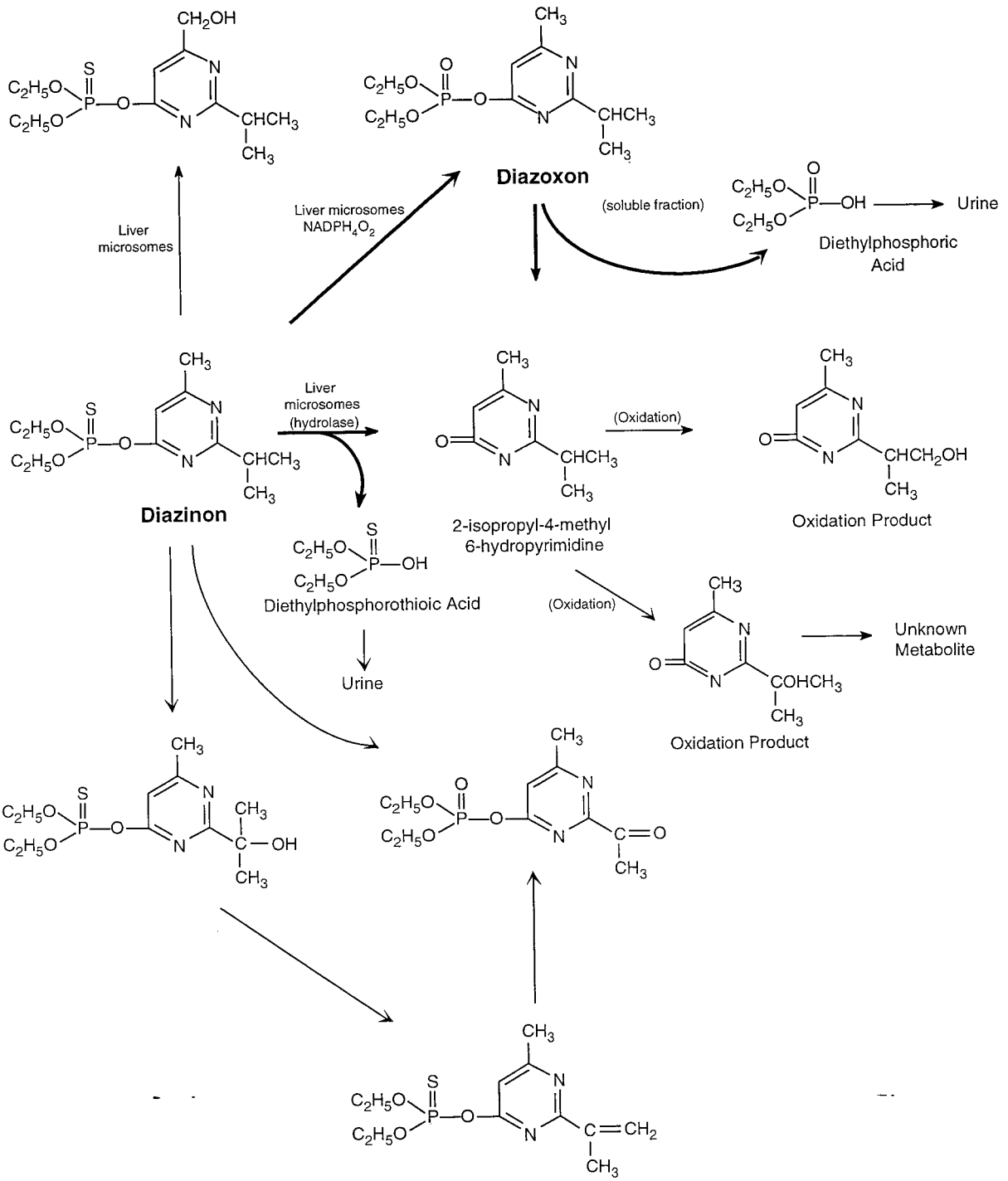
2.3.4.1 Inhalation Exposure

No studies were located regarding excretion of diazinon after inhalation exposure in animals or humans.

2.3.4.2 Oral Exposure

Following a single oral dose of 4.0 mg/kg 2-pyrimidinyl ring-labeled and 4-pyrimidinyl ring-labeled [^{14}C] diazinon to rats, approximately 50% of the dose was excreted within 12 hours of dosing (Miicke et al. 1970). Sixty-nine to 80% of the radioactivity was recovered in the urine and 18-25% was excreted in the feces. Only 5.6% of an ethyl-[^{14}C]diazinon dose was recovered as $^{14}CO_2$ in expired air. No $^{14}C0_2$ was expired from rats given an oral dose of 2-[^{14}C] or 4-[^{14}C]pyrimidine diazinon

Figure 2-3. Proposed Mammalian Metabolic Pathway for Diazinon



Source: Adapted from Aizawa 1989; Yang et al. 1971

indicating that complete degradation of the pyrimidine ring did not take place. Traces of unchanged diazinon were recovered in the feces. Three of the unidentified metabolites recovered in the urine and feces of treated rats accounted for 70% of the total administered dose. The half-life of the ^{14}C-ring labeled diazinon was 12 hours while that of [ethyl-^{14}C]diazinon was 7 hours (Mi.icke et al. 1970). Recovery of radioactivity in the urine of female Beagle dogs 24 hours after receiving a single oral dose of [^{14}C]diazinon was 85% (53% water soluble fraction, and 2 metabolites which no longer had a phosphorothioate group, comprising 10 and 23%). No diazinon was detected in the feces (Iverson et al. 1975). Following oral administration of diazinon to lactating goats, diethyl phosphorothioate was detected in the urine but not in the milk (Mount 1984).

2.3.4.3 Dermal Exposure

Human volunteers were exposed for 24 hours to 2-pyrimidinyl ring-labeled [^{14}C]-diazinon applied to either the forearm or abdomen in either an acetone solution or a lanolin wool grease at doses of approximately 15-20 μg/dose for each application method to test the percutaneous absorption of diazinon. Daily complete void urine samples were collected and analyzed for levels of radioactivity for 7 days after dosing. Percutaneous absorption, calculated from the amount of radioactivity present in the urine, was reported as 2.9-3.85% of the administered dose (Wester et al. 1993).

2.3.4.4 Other Exposure

Following an intravenous injection of [ethyl-^{14}C]diazinon to female Beagle dogs, approximately 58% of the radioactivity was recovered in the urine within 24 hours as diethyl phosphorothioic acid (42%) and diethyl phosphoric acid (16%). No intact diazinon was excreted (Iverson et al. 1975).

2.4 MECHANISMS OF ACTION

Diazinon toxicity results predominantly from the inhibition of acetylcholinesterase in the central and peripheral nervous system. The enzyme is responsible for terminating the action of the neurotransmitter, acetylcholine, in the synapse of the pre- and post-synaptic nerve endings or in the neuromuscular junction. However, the action of acetylcholine does not persist long as it is hydrolyzes by the enzyme, acetylcholinesterase, and rapidly removed. As an anticholinesterase organophosphate, diazinon inhibits acetylcholinesterase by reacting with the active site to form a stable phosphorylated

complex which is incapable of destroying acetylcholine at the synaptic gutter between the pre- and post-synaptic nerve endings or neuromuscular junctions of skeletal muscles resulting in accumulation of acetylcholine at these sites. This leads to continuous or excessive stimulation of cholinergic fibers in the post-ganglionic parasympathetic nerve endings, neuromuscular junctions of the skeletal muscles, and cells of the central nervous system that results in hyperpolarization and receptor desensitization. These cholinergic actions involving end organs (heart, blood vessels, secretory glands), innervated by fibers in the postganglionic parasympathetic nerves, result in muscarinic effects. Muscarinic effects are manifested as miosis, excessive glandular secretions (salivation, lacrimation, rhinitis), nausea, urinary incontinence, vomiting, abdominal pain, diarrhea, bronchoconstriction or bronchospasm, increased bronchosecretion, vasodilation, bradycardia, and hypotension. Nicotinic effects are due to accumulation of acetylcholine at the skeletal muscle junctions and sympathetic preganglionic nerve endings. Nicotinic effects are manifested as muscular fasciculations, weakness, mydriasis, tachycardia, and hypertension. The central nervous system effects are due to accumulation of acetylcholine at various cortical, subcortical, and spinal levels (primarily in the cerebral cortex, hippocampus, and extrapyramidal motor system). The central nervous system effects are manifested as respiratory depression, anxiety, insomnia, headache, restlessness, tension, mental confusion, loss of concentration, apathy, drowsiness, ataxia, tremor, convulsion, and coma (Klaassen et al. 1986; Williams and Burson 1985). Although diazinon directly inhibits acetylcholinesterase, its oxidation product, diazoxon (Iverson et al. 1975; Yang et al. 1971) formed in the liver, is an even more potent inhibitor of the enzyme (Davies and Holub 1980a, 1980b; Edson and Noakes 1960; Enan et al. 1982; Harris et al. 1969; Rajendra et al. 1986; Takahashi et al. 1991).

The primary cause of death in acute diazinon poisoning is a depression of the neurons in the brainstem (medulla), collectively known as the respiratory center, resulting in loss of respiratory drive or, in the case of managed treatment, cardiac failure due to electrical impulse or beat conduction abnormalities in cardiac muscles (fatal arrhythmias). Other effects, such as bronchoconstriction, excessive bronchial secretions, and paralysis of the respiratory muscles (intercostal muscles and diaphragm) may also contribute to respiratory insufficiency and death. Thus, death results from loss of respiratory drive and paralysis of the respiratory muscles, or cardiac failure, or both, with attendant asphyxia or cardiac arrest (Klaassen et al. 1986; Shankar 1967, 1978; Williams and Burson 1985).

2.5 RELEVANCE TO PUBLIC HEALTH

Diazinon is an insecticide with broad-based use in both agriculture and in control of pests in residential dwellings, gardens, and on household pets. Like most organophosphorus insecticides, diazinon and its active metabolite diazoxon are rapidly hydrolyzed to non-toxic products. By far the greatest potential for significant exposure to this compound is found in occupational settings (i.e., manufacture and application of diazinon). The most common exposure scenario for the general population comes from home use of the product to control garden pests, application to animals in the form of shampoos, and residual contamination following indoor application for the control of insects. In all of the studies reviewed which reported an association between diazinon exposure and adverse health effects in humans, the levels of known or potential exposure to diazinon suggested direct contact and use of the compound or exposure to a treated environment. No association has been reported between diazinon toxicity and low level environmental contamination.

As an anticholinesterase organophosphate, the principal toxic effect of diazinon in humans and laboratory animals is inhibition of acetylcholinesterase (Coye et al. 1987; Davies and Holub 1980a, 1980b; Edson and Noakes 1960; Enan et al. 1982; Harris et al. 1969; Rajendra et al. 1986; Takahashi et al. 1991; Wecker et al. 1985). Inhibition of acetylcholinesterase results in the accumulation of acetylcholine at acetylcholine receptors leading to cholinergic responses in the peripheral (muscarinic and nicotinic) and central nervous system and neuromuscular junctions. Severe acetylcholinesterase inhibition often leads to cholinergic symptoms in humans and laboratory animals which include excessive glandular secretions (salivation, lacrimation, rhinitis), miosis, bronchoconstriction, vasodilation, hypotension, diarrhea, nausea, vomiting, urinary incontinence, and bradycardia. Tachycardia, mydriasis, fasciculations, cramping, twitching, muscle weakness, and muscle paralysis are associated with nicotinic receptor stimulation. Central nervous system toxicity includes respiratory depression, anxiety, insomnia, headache, apathy, drowsiness, dizziness, loss of concentration, confusion, tremors, convulsions, and coma. In non-fatal exposures, the effects are transient and recovery is rapid and complete following cessation of exposure (Bichile et al. 1983; Hata.et al. 1986; Klemmer et al. 1978; Reichert et al. 1977; Wadia et al. 1974; Wedin et al. 1984). In sufficiently high doses, death may result in some cases (Kabrawala et al. 1965; Limaye 1966; Poklis et al. 1980). These effects usually occur within a few minutes to 24 hours after dosing, depending on the extent of exposure.

Minimum Risk Levels (MRLs) for Diazinon.

Inhalation MRLs.

- An MRL of 0.009 mg/m^3 has been derived for intermediate-duration inhalation exposure (15-364 days) to diazinon.

This MRL is based on a NOAEL of 0.46 mg diazinon/m^3 for brain acetylcholinesterase inhibition (the target of diazinon toxicity) observed in a 21-day study in hybrid rats (Hartman 1990). Groups of rats (10 of each sex) were exposed to control air or air containing 4 different concentrations of aerosolized diazinon (0.05, 0.46, 1.57, or 11.6 mg/m^3) for 6 hours a day, 5 days a week for 3 weeks. Compared to control, no clinical signs of organophosphate neurotoxicity were observed or any effect on survival or body weight. Histopathology of the nasal tract and lungs was normal in all groups, as was the spleen, heart, liver, kidney, and adrenal gland (examined only in the 11.6 mg/m^3 groups). A doseresponse relationship existed for both erythrocyte and brain acetylcholinesterase in the female rats, and a NOAEL of 0.46 mg diazinon/m^3 was identified. This level was converted to a Human Equivalent Concentration to adjust for the different penetration of aerosols in the human and rat respiratory tract (see Appendix A) and also adjusted for intermittent versus constant exposure. An uncertainty factor of 30 (3 for animal-to-human extrapolation and 10 for human variability) was applied resulting in a MRL of 0.009 mg/m^3.

Acute- and chronic-duration inhalation exposure MRLs for diazinon were not derived because of a lack of suitable studies in the literature. Since diazinon is not volatile, inhalation exposure near toxic waste sites is probably less likely than oral or dermal exposure. However, the potential risk of adverse health effects from inhalation exposure to diazinon cannot be assessed without information on the levels actually present in the air around the site. The MRL level of 0.009 mg diazinon/m^3 should be protective for individuals living near waste sites. NIOSH has recommended a Permissible Exposure Level (PEL) of 0.1 mg diazinon/m^3 to protect the health of individuals who regularly use diazinon in their work; the MRL level of 0.009 mg/m^3 is approximately l00-fold lower.

Oral MRLs.

- . An MRL of 0.0002 mg/kg/day has been derived for intermediate-duration oral exposure (15-364 days) to diazinon.

This MRL is based on a NOAEL of 0.02 mg/kg/day for brain acetylcholinesterase inhibition in Beagle dogs receiving diazinon in their food daily for 13 weeks (Barnes 1988). Groups of 4 dogs of each sex consumed 0, 0.0034, 0.02, 5.9, or 10.9 mg diazinon/kg/day (males) or 0, 0.0037, 0.021, 5.6, or 11.6 mg diazinon/kg/day (females) for 13 weeks. No deaths occurred during the study. Emesis and diarrhea were reported in all groups, but the effects were not dose-related. No histopathological evidence of treatment-related effects were seen in brain, spinal cord, peripheral nerve, or optic nerve. A dose-response for brain acetylcholinesterase inhibition was demonstrated in the two highest dose groups in both sexes and a NOAEL of 0.02 mg/kg/day was observed. The MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).

An MRL for acute-duration oral exposure was not derived because information on the purity of the diazinon used was unavailable in most of the studies for this duration of exposure. A NOAEL of 0.05 mg/kg/day for brain acetylcholinesterase inhibition was identified in a chronic-duration study on diazinon in rats (Kirchner 1991). However, the chronic-duration MRL derived from this study would be 0.0005 mg/kg/day. Since the intermediate-duration MRL (0.0002 mg/kg/day) would be more protective, it was the only one derived. This MRL should be protective for individuals living near hazardous waste sites. The most likely means by which oral exposure to diazinon would occur at a waste site would be via contamination of drinking water or the unintentional ingestion of contaminated groundwater or soil. Measurement of diazinon levels in these media would be necessary to assess whether any harmful effects might occur. It is known from studies in rats (Kirchner 1991; Singh 1988) and dogs (Barnes 1988) that oral exposure at approximately loo-fold higher than the MRL level is necessary before inhibition of the most sensitive biomarker for diazinon exposure (serum - - -. cholinesterase) can be demonstrated. Serum cholinesterase inhibition alone has never been associated with toxicity.

Death. In most cases of diazinon-related deaths in humans, the amount of the insecticide ingested is not known. Thus, an estimate of the minimally lethal dose is not possible. One study reported that

the ingestion of approximately 293 mg/kg of a 10% diazinon formulation was fatal in a 54-year-old woman (Wecker et al. 1985). In rats, the oral LD_{50}, for technical diazinon for both sexes ranges from 76 to 300 mg/kg (Enan et al. 1982; Gaines 1960, 1969; Takahashi et al. 1991), which is in the dose range estimated to be fatal in other human reports (Wecker et al. 1985). Long-term exposure to 10 mg/kg/day diazinon in dogs caused 30% mortality after 8 months (Earl et al. 1971).
No reports of human deaths resulting from dermal exposure to diazinon were located, but evidence from non-lethal human data and animal lethality studies indicates that human lethality by these routes of exposure is unlikely (Edson and Noakes 1960; Gaines 1960; Lee 1989; Noakes and Sanderson 1969).

Systemic Effects.

Respiratory Effects. In humans and laboratory animals, acute exposure to sufficiently high doses (as found in accidental ingestion or suicide attempts) led to respiratory distress as a component of the spectrum of the symptoms of cholinergic effects resulting from acetylcholinesterase inhibition. In a human study, acute oral doses of diazinon induced pulmonary distress in the poisoned victims by causing bronchoconstriction, increased bronchial secretions, pulmonary edema, active hyperemia, hypostatic congestion, and pneumonia (Poklis et al. 1980; Shankar 1967). A 56-year-old female gardener, dermally exposed to spilled diazinon of unknown purity, developed pulmonary edema with bilateral lung crepitations and tachypnea (Lee 1989). In rats, single oral doses of 50-700 mg/kg diazinon produced pulmonary inflammation, vascular congestion, venous stasis, and occasional extensive pneumonitis (Boyd and Carsky 1969). The most common cause of death in that study was respiratory failure, which is in keeping with the postulated mechanism of diazinon-related deaths.

Cardiovascular Effects. A 56-year-old female gardener, dermally exposed to spilled diazinon of unknown purity, developed sinus tachycardia with no evidence of infarction and showed increased cardiac enzyme (serum glutamate oxalate transaminase, total lactate dehydrogenase creatine phosphokinase) levels. The victim was diagnosed on discharge with acute left ventricular failure (Lee 1989). In dogs, diazinon was associated with an absence of pericardial fat (Earl et al. 1971). Additionally, diazinon-related elevations in serum LDH were also found. This is a nonspecific response that may be suggestive of either cardiac or skeletal muscle damage, or some other unknown biological activity (Earl et al. 1971).

Gastrointestinal Effects. Acute pancreatitis (as well as other gastrointestinal disturbances) appears to be a component of high acute diazinon intoxication in humans, manifesting as a secondary effect of the spectrum of cholinergic reactions by some unknown mechanism. Oral exposure to diazinon has been shown to cause gastrointestinal irritation, ulceration, and congested stomach mucosa with submucosal petechial hemorrhage in humans (Poklis et al. 1980). A 16-year-old female who drank an estimated 1.5 mg/kg of a diazinon formulation (Tik-20) developed pancreatitis after being treated for cholinergic manifestations. The pancreatic effects may well have been secondary to the diazinoninduced cholinergic manifestations (Dagli et al. 1981). Acute pancreatitis was also found in two children poisoned with diazinon (Weizman and Sofer 1992). Dermal exposure to diazinon in humans also produced gastric irritation and signs of acute pancreatitis which included abdominal colic, diarrhea, nausea, vomiting, and epigastric pain, as well as elevated serum amylase and urinary diastase levels) (Lee 1989). In animal studies, oral exposure to high acute diazinon doses has resulted in gastrointestinal tract histopathology as well (Boyd and Carsky 1969).

Hematological Effects. Diazinon exposure is not likely to produce adverse effects on the blood and bone marrow in humans. Hematological analysis performed on samples from 5 individuals (3 males, 2 females) who intentionally ingested 60-180 mL of 25% diazinon solution (estimated to deliver a dose of 240-400 mg/kg for males and 509-986 mg/kg for females) found that leucocyte counts, hemoglobin, and hematocrit were all within normal ranges (Klemmer et al. 1978). Although two female gardeners, dermally exposed to spilled diazinon, developed hypokalemia and leucocytosis, these symptoms were not observed in any other human study with diazinon. Furthermore, the purity of the diazinon in this exposure is unknown; therefore, these effects may be due to impurities or other chemical compounds in the formulation of the diazinon sample (Lee 1989). In animal studies, male rats acutely exposed to oral doses of up to 4.4 mg/kg diazinon exhibited reduced platelet counts and fibrinogen activity; the activity of other clotting components (prothrombin, partial thromboplastin, factor II, factor V, and factor X) was increased (Lox 1983). Since fibrinogen and factors II, V, and X are synthesized in the liver, however, the changes in clotting factor activity may be the result of diazinon-induced hepatic toxicity. Similar results were noted in a 14-day study using a single dose of 52 mg/kg in female rats (Lox 1987) and a 6-month study using a single dose of 0.18 mg/kg in female rats (Lox and Davis 1983). However, these results may not be reliable because the factor-deficient substrates used to measure the rat plasma activities were of human origin and, therefore, the observations might reflect some degree of cross-species differences. In addition to affecting clotting, oral exposure to diazinon in dogs has been shown to cause anemia, perhaps, by directly affecting the

bone marrow since elevations in the myeloid/erythroid ratio were found (Earl et al. 1971). In pigs, intermediate-duration exposure to diazinon also caused anemia, reticulocytopenia, and an elevated myeloid/erythroid ratio (Earl et al. 1971). But there is no evidence that similar effects occur in humans.

Musculoskeletul Effects. Diazinon exposure is not likely to produce irreversible adverse effects on the muscle and skeleton in humans. No musculoskeletal effects were reported in humans from exposure to diazinon by the oral route. One study reported mild pathologic changes in the intercostal muscles of a man who died after inhaling a large dose of diazinon. Acetylcholinesterase activity in that man was found to be 50% lower than normal, which is consistent with organophosphate (diazinon) exposure (Wecker et al. 1985). In animals, acute oral exposure to diazinon in dogs elevated serum LDH. This is a nonspecific response that may be suggestive of either cardiac or skeletal muscle damage or some other unknown biological activity (Earl et al. 1971).

Hepatic Effects. No hepatic effects were reported in humans from exposure to low doses of diazinon by any route. Hepatic effects reported in human studies were associated with acute high levels of diazinon such as are found in suicide attempts (Limaye 1966). The acute- and intermediate-duration oral exposure to diazinon in animals reduced the activity of a variety of hepatic enzymes (Anthony et al. 1986; Mihara et al. 1981). Liver pathology has also been observed in animals following chronic oral exposure to diazinon. The pathological changes reported in one study included fatty infiltrations, parenchymal atrophy, hepatocyte dissociation, mild cirrhosis, and focal necrosis (Earl et al. 1971). The intermediate dermal exposure to diazinon increased fecal porphyrin excretion, indicating that diazinon may interfere with hepatic metabolism of porphyrins (Bleakely et al. 1979). However, it is not certain that similar effects would occur in humans following diazinon exposure.

Renal Effects. The evidence from humans and laboratory animals exposed to diazinon indicates that adverse renal effects may occur in humans after exposure to high acute doses of diazinon. Oral diazinon exposure in humans is associated with renal structural damage and altered kidney function (Poklis et al. 1980; Wedin et al. 1984); however, the effects may have been due to impurities or solvents present in the diazinon formulations. The deliberate ingestion of approximately 293 mg/kg of a diazinon formulation (“FERTI-LOME” bagworm spray; 10% diazinon) caused renal congestion, renal cortical submucosal petechiae, and ecchymoses (Poklis et al. 1980). In animals, single oral doses of diazinon ranging from 50 to 700 mg/kg produced dose-dependent renal toxicity (Boyd and Carsky

1969). The effects included biochemical alterations (aciduria and alkalinuria, hematuria, glycosuria, proteinuria, and hematuria) and renal structural damage (tubular swelling and capillary loop congestion). The toxicity was increased by protein deprivation prior to treatment (Boyd and Carsky 1969).

Endocrine Effects. Acute pancreatitis appears to be a component of severe acute diazinon intoxication in humans, manifesting as a secondary effect of the spectrum of cholinergic reactions by some unknown mechanism. Acute pancreatitis, as determined by blood chemistry analysis, was found in two children poisoned with an unknown quantity or formulation of diazinon (Weizman and Sofer 1992). A 16-year-old human female also exhibited pancreatitis following ingestion of 1.5 mg/kg of a diazinon formulation (Tik-20) (Dagli et al. 1981). Two female gardeners, 56 and 48 years old, dermally exposed to spilled diazinon of unknown purity, developed signs of acute pancreatitis which included abdominal colic, diarrhea, nausea, vomiting, and epigastric pain, as well as elevated serum amylase and urinary diastase levels (Lee 1989).

Dermal Effects. Dermal exposure to diazinon resulted in contact dermatitis in farm workers (Matsushita et al. 1985). But in another human dermal exposure study, diazinon failed to elicit an irritation response (Lisi et al. 1987). In laboratory animal studies, diazinon was also not irritating to guinea pigs in a 24-hour occluded skin patch test (Matsushita et al. 1985).

Body Weight Effects. Body weight loss after exposure to diazinon is not likely to be a health effect of concern in humans. Although significant loss of body weight was observed in dogs given oral diazinon doses of ≥10 mg/kg, no clear dose-response for this effect was observed in the study. The observed body weight reductions were probably a result of reduced food intake, emesis, diarrhea, generalized emaciation, and anorexia reported in the study (Earl et al. 1971). No body weight reductions were reported in the available human studies.

Immunological and Lymphoreticular Effects. The potential for diazinon exposure to induce adverse immunological/lymphoreticular effects in humans is not certain. Acute oral diazinon exposure in rats has been shown to reduce spleen weight by 35%, cause splenic red pulp contraction, decrease thymus weight, and induce thymic atrophy (Boyd and Carsky 1969). The intermediate oral administration of diazinon to dogs caused splenic shrinking (Earl et al. 1971). The splenic atrophy reported in this study may be a result of the generalized emaciated condition of the dog due to

diarrhea, emesis, and anorexia, as reported in the study. It is not certain that similar effects would occur in humans following diazinon exposure.

Human subjects showed allergic interaction between the fungicide benomyl and diazinon following dermal exposure to these insecticides (Matsushita and Aoyama 1981). In animal dermal exposure studies, guinea pigs displayed an allergic interaction between the fungicide benomyl and diazinon (Matsushita and Aoyama 1981). Similarly, diazinon caused delayed contact hypersensitivity 24 and 48 hours after challenge in the guinea pig maximization test (Matsushita et al. 1985). When cross-sensitization was tested using a challenge of 0.2 or 2% benomyl insecticide, allergenicities were grade I (0%) and grade III (30%), respectively (Matsushita et al. 1985).

Neurological Effects. As an anticholinesterase organophosphate, diazinon inhibits blood and tissue acetylcholinesterase in mammals (Dagli et al. 1981; Davies and Holub 1980a, 1980b; Edson and Noakes 1960; Enan et al. 1982; Harris et al. 1969; Rajendra et al. 1986; Takahashi et al. 1991). In high acute doses, diazinon causes severe acetylcholinesterase inhibition that often leads to cholinergic signs and symptoms, manifest as reversible neuromuscular dysfunction when treated or when exposure is terminated. These manifestations include muscarinic effects (bronchoconstriction, increased bronchosecretion, nausea and vomiting, diarrhea, bradycardia, hypotension, miosis, urinary incontinence), nicotinic effects (tachycardia, hypertension, muscular twitching and weakness, fasciculation, cramping), and central nervous system effects (anxiety, apathy, depression, giddiness, drowsiness, insomnia, nightmares, headaches, confusion, ataxia, depressed reflex, seizure, respiratory depression, coma). In sufficiently high exposures (such as are found in suicide attempts or accidental ingestions) death from respiratory failure may result without timely treatment intervention (Bichile et al. 1983; Dagli et al. 1981; Hata et al. 1986; Kabrawala et al. 1965; Klemmer et al. 1978; Reichert et al. 1977; Schenker et al. 1992; Shankar 1967, 1978; Wadia et al. 1974; Wedin et al. 1984). Similarly, inhalation exposure to high acute doses of diazinon may result in cholinergic signs and a variety of symptoms that stem predominately from acetylcholinesterase inhibition. In acute inhalation poisoning reports where bo_th inhalation and dermal exposure may have occurred, victims exhibitedneurological symptoms or cholinergic signs and symptoms that often resulted in death from respiratory or cardiac failure (Adlakha et al. 1988; Rayner et al. 1972; Richter et al. 1992; Soliman et al. 1982). The cholinergic manifestations of high acute exposure to diazinon have also been reported in animals and include anorexia, ataxia, epistaxis, tremors, listlessness, gasping, convulsions, tachypnea, dyspnea, prostration, fasciculations, twitches, exophthalmos, diarrhea, salivation, diuresis, lacrimation,

prostration, Straub tail reflex, and hypothermia (Boyd and Carsky 1969; Earl et al. 1971; Takahashi et al. 1991; Williams et al. 1959). Protein deficiency may potentiate diazinon-induced toxicity (Takahashi et al. 1991), perhaps by limiting the hepatic synthesis of the enzymes necessary to metabolize diazinon.

Longer-term exposure to lower diazinon doses may lead to the development of subtle behavioral/ cognitive ability deficits. A case study of 99 adult humans (67 males and 32 females) who had been potentially exposed to diazinon granules occupationally for 8 hours a day for 39 days during an insecticide application program reported manifestations of decreased post-shift symbol-digit speed and pattern memory accuracy (Maizlish et al. 1987). In one animal study, mice exposed to diazinon *in utero* exhibited signs of behavioral/functional deficits (as manifested by delayed contact placing and sexual maturity); they also exhibited endurance and coordination deficits: decreased performance on neuromuscular function tests (rod cling and inclined plane), slower running speed in a Lashley II maze, and reduced swimming endurance. These effects have been postulated to occur either indirectly through diazinon impairment of placental transport of nutrients or maternal regulation of fetal growth, or directly via antagonism to cholinergic development of the fetus (Spyker and Avery 1977). Thus, prolonged low-level diazinon exposure in adult humans or *in utero* exposure may result in functional deficits in otherwise normal humans that can only be detected by systematic behavioral evaluation.

Reproductive Effects. Diazinon exposure is not likely to result in any significant reproductive effects in humans. No information was located on the reproductive effects of diazinon exposure in humans. Only three animal studies on the reproductive effects of diazinon exposure were located. The first study in rats found that oral diazinon exposure increased litter size (Green 1970), while a second rat study reported significant reduction ($p<0.05$) in litter size at oral maternal diazinon doses of 0.18 and 9 mg/kg/day (Spyker and Avery 1977). A third study, a 4-generation study which used only 3 dogs per sex, reported a dose-response testicular atrophy and arrested spermatogenesis in males in the fourth generation of male dogs (Earl et al. 1971).

Developmental Effects. Diazinon exposure is not likely to result in significant developmental effects in humans. No information on the developmental effects of diazinon in humans from inhalation, oral, or dermal exposure was located. However, there is limited evidence that prenatal exposure to low levels of diazinon may result in functional deficits in otherwise normal animals that can only be detected by systematic behavioral evaluation (Spyker and Avery 1977). These slight

neuromuscular deficits might occur either indirectly through diazinon impairment of placental transport of nutrients or maternal regulation of fetal growth, or directly via antagonism to cholinergic development of the fetus. Furthermore, several studies in laboratory animals report developmental anomalies in rats. One of these studies exposed rats during gestation to diazinon and concluded that prenatal diazinon exposure caused focal forebrain abnormalities typified by dense aggregations of atypical chromatin-containing cells in offspring. These offspring displayed slight neuromuscular deficit as manifested by decreased performance on rod clinging and inclined plane tests (Spyker and Avery 1977). Diazinon has also been reported to increase the number of fetal resorptions and increase the incidence of visceral (hydroureter, hydronephrosis) and skeletal (rudimentary, short, wavy rib; hypocalcification) variations at moderate doses (Dobbins 1967) and cause offspring growth retardation in rats in relatively low doses (Green 1970). However, the effects reported in these studies did not show dose-response. In addition, other studies conducted with hamsters and rabbits failed to result in any developmental effect in the offspring of these hamsters and rabbits. The maternal doses used in one of the studies that produced negative developmental effects in rabbits were sufficiently high to induce severe cholinergic reaction in the maternal rabbits (Robens 1969).

Genotoxic Effects. The genotoxic effects of diazinon in humans are not known because existing human data are inadequate and the results of *in vitro* laboratory testing data in mammalian cells and microorganisms are equivocal. Several reports that described potentially chronic occupational inhalation and dermal exposures to multiple insecticides, including diazinon, associated these exposures with increased incidence of chromosomal aberrations and increased sister chromatid exchanges in peripheral blood lymphocytes (De Ferrari et al. 1991; Kiraly et al. 1979; See et al. 1990). However, it is not possible to attribute these genotoxic effects exclusively to diazinon because the workers were concurrently or sequentially exposed to up to 80 different insecticides in unknown amounts for variable durations as indicated in these reports. In the laboratory, the mutagenicity of diazinon has been studied in a variety of test systems, with mixed results.. *In vitro* test results show that diazinon was positive for gene mutations in the *Salmonella typhimurium* test assay with metabolic activation (Wong et al. 1989) and in the mouse lymphoma cell forward mutation assay without metabolic activation (McGregor et al. 1988). The compound was also positive for chromosomal aberrations in Chinese hamster cells with metabolic activation (Matsuoka et al. 1979). But in other tests, diazinon was negative for gene mutations in the *Salmonella. typhimurium* test assay (Marshall et al. 1976) and in the recassay utilizing strains of *Bacillus subtilis* (Shirasu et al. 1976). Both of these studies were conducted without metabolic activation. Tests for sister chromatid exchange in Chinese hamster V79 cells, both

with and without metabolic activation (Chen et al. 1982), and for chromosomal aberrations in human peripheral blood lymphocytes (Lopez et al. 1986), were also negative. Table 2-4 lists genotoxic effects of diazinon *in vitro*.

Cancer. There is no specific evidence from epidemiological studies that diazinon causes cancer in humans. Several studies have reported an increased incidence of cancers (brain tumors, Hodgkin's lymphoma, multiple myelomas) in humans who were concurrently or sequentially exposed to a number of insecticides, including diazinon (Cantor et al. 1992; Davis et al. 1993; Morris et al. 1986). However, it is not possible to attribute the increase in cancer incidence exclusively to diazinon exposure. Consequently, while the findings in these studies suggest an elevated risk for the cancers from high exposure to insecticides, in general, the data are far too limited to be used to evaluate the potential for diazinon to cause cancer in humans.

The evidence from animal studies suggest that diazinon exposure is not likely to cause cancers in humans. While not designed as a cancer bioassay, in a recent study where rats (groups of 20-30) were orally exposed to diazinon (Kirchner 1991) for 98 weeks, histopathology of some 30-40 different tissues showed no treatment-related increase in neoplasms. No carcinogenic effects in laboratory animals following inhalation or dermal exposure to diazinon were reported in any of the located studies. A short-term animal cancer bioassay conducted in mice by intraperitoneal administration of diazinon was associated with increased incidence of lung cancers in a strain of mice (A/St) that are naturally predisposed to this type of cancer (Maronpot et al. 1986). The positive conclusion in this study regarding the potential for diazinon to produce animal cancers and its relevance to human risk from cancers is questionable because of the predisposition of the strain of mice used in the study to develop the type of cancers observed in the study as well as the route of diazinon administration used in the study.

The National Cancer Institute (NCI) concluded that diazinon was not carcinogenic in either rats or mice following chronic bioassays in Fischer 344 rats and B6C3F_1mice (NCI 1979).

2.6 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility

Table 2-4. Genotoxicity of Diazinon *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Reverse mutation				
B. subtilis (rec assay)	Gene mutation	ND	–	Shirasu et al. 1976
S. typhimurium	Gene mutation	+	–	Wong et al. 1989
S. typhimurium	Gene mutation	ND	–	Marshall et al.1976
Eukaryotic cells:				
Human peripheral blood lymphocytes	Chromosomal aberration	ND	–	Lopez et al. 1986
Mouse lymphoma cells	Gene mutation	ND	+	McGregor et al. 1988
Chinese hamster cells	Sister chromatid exchange	–	–	Chen et al. 1982
Chinese hamster cells	Chromosomal aberrations	+	–	Matsuoka et al. 1979

– = negative result; + = positive result; ND = Not done

(NAS/NRC 1989). Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to diazinon are discussed in Section 2.6.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by diazinon are discussed in Section 2.6.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic-or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.8, Populations That Are Unusually Susceptible.

2.6.1 Biomarkers Used to Identify or Quantify Exposure to Diazinon

Diazinon is rapidly absorbed from the gastrointestinal tract and widely distributed throughout the body in both humans (Poklis et al. 1980) and animals (Janes et al. 1973; Mticke et al. 1970). No human or animal studies have reported the presence of unchanged diazinon in the urine following exposure. Traces of unchanged diazinon have been detected in animal feces following exposure (Mucke et al. 1970). Diazinon undergoes biotransformation to a variety of polar metabolites which have been detected in the urine and feces of animals. Urinary and fecal excretion of 2-isopropyl-4-methyl- 6-hydroxypyrimidine, diethyl phosphosphorothioic acid, and diethyl phosphoric acid have been reported following exposure of animals to diazinon (Aizawa 1989; Iverson et al. 1975; Machin et al. 1975; Mount 1984; Mticke et al. 1970; Yang et al. 1971) while diethyl phosphosphorothioic acid and diethyl phosphoric acid have been detected in the urine of exposed insecticide applicators (Maizlish et al. 1987). Analysis of blood samples for the presence of these metabolites represents a potential means of assessing exposure; however, only 2-isopropyl-4-methyl-6-hydroxypyrimidine is specific for diazinon. Analysis of urine samples for metabolic products provides a non-invasive method for detecting exposure. As diazinon is rapidly metabolized and excreted from the body, urinary and fecal metabolite analysis is useful only in the evaluation of recent exposures. There are no studies which report a quantitative association between metabolite levels and exposure to diazinon in humans. Therefore, these biomarkers are only indicative of exposure and are not useful for dosimetric analysis.

2.6.2 Biomarkers Used to Characterize Effects Caused by Diazinon

The major action resulting from human exposure to diazinon is the inhibition of cholinesterase activity (refer to Section 2.4 for discussion). Two pools of cholinesterases are present in human blood; acetylcholinesterase in erythrocytes and serum cholinesterase (sometimes referred to as pseudocholinesterase or butyrlcholinesterase) in plasma. Acetylcholinesterase, present in human erythrocytes, is identical to the enzyme present in neural tissue (the target of diazinon action) while serum cholinesterase has no known physiological function. Inhibition of both forms of cholinesterase have been associated with exposure to diazinon in humans and animals (Coye et al. 1987; Edson and Noakes 1960: Soliman et al. 1982). Inhibition of erythrocyte, serum, or whole blood cholinesterase may be used as a marker of exposure to diazinon. However, cholinesterase inhibition is a common action of anticholinesterase compounds such as organophosphates (which include diazinon) and carbamates. In addition, a wide variation in normal cholinesterase values exists in the general population, and there are no studies which report a quantitative association between cholinesterase activity levels and exposure to diazinon

in humans. Thus, cholinesterase inhibition is not a specific biomarker of effect for diazinon exposure, but is indicative only of effect, and not useful for dosimetric analysis.

It should be noted that serum cholinesterase activity has been reported to be a more sensitive marker for diazinon exposure than erythrocyte acetylcholinesterase (Endo et al. 1988; Hayes et al. 1980). In light of this, it has been suggested that in the absence of baseline values for cholinesterase activity, sequential post-exposure cholinesterase analyses be used to confirm a diagnosis of organophosphate poisoning (Coye et al. 1987).

In combination with analysis of reductions in the level of cholinesterase activity, the manifestations of severe diazinon poisoning, clinically characterized by a collection of cholinergic signs and symptoms (which my cause dizziness, fatigure, tachycardia, or bradycardia, miosis, and vomiting) (Bichile et al. 1983; Dagli et al. 1981; Hata et al. 1986; Kabrawala et al. 1965; Klemmer et al. 1978; Reichert et al. 1977; Wadia et al. 1974; Wedin et al. 1984) are useful biomarkers of effect for identifying poisoned victims of diazinon. These manifestations are also not specific to diazinon but to anticholinesterase compounds (such as organophosphates and carbamates) in general.

For more information on biomarkers for renal and hepatic effects of chemicals see ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage (1990) and for information on biomarkers for neurological effects see OTA (1990).

2.7 INTERACTIONS WITH OTHER CHEMICALS

The toxicity of diazinon may be affected by other substances. Some chemicals may increase the toxicity of diazinon in an additive manner. Anticholinesterase organophosphates and carbamates would be expected to act in an additive manner with diazinon with respect to its potential to induce cholinergic toxicity.

Other chemicals may interfere with the toxicity of diazinon indirectly by influencing its metabolism through their actions on drug metabolizing enzymes. The duration and intensity of action of diazinon are largely determined by the speed at which it is metabolized in the body by the oxidative and hydrolytic enzymes of the liver. More than 200 drugs, insecticides, carcinogens, and other chemicals are known to induce the activity of liver microsomal drug-metabolizing enzymes. The characteristic

biological actions of these chemicals are highly varied. Although there is no relationship between their actions or structures and their ability to induce enzymes, most of the inducers are lipid soluble at physiological pH. These inducers of the MFO system include the following classes of drugs: hypnotic and sedatives (barbiturates, ethanol); anesthetic gases (methoxyflurane, halothane); central nervous system stimulators (amphetamine); anticonvulsants (diphenylhydantoin); tranquilizers (meprobamate); antipsychotics (triflupromazine); hypoglycemic agents (carbutamide); anti-inflammatory agents (phenylbutazone); muscle relaxants (orphenadrine); analgesics (aspirin, morphine); antihistaminics (diphenhydramine); alkaloids (nicotine); insecticides (chlordane, DDT, BHC, aldrin, dieldrin, heptachlorepoxide, pyrethrins); steroid hormones (testosterone, progesterone, cortisone); and carcinogenic polycyclic aromatic hydrocarbons (3-methylcholanthrene, 3,4-benzpyrene) (Klaassen et al. 1986; Williams and Burson 1985).

Thus, exposure to any of these enzyme inducers concurrent with or after exposure to diazinon may result in accelerated bioactivation to the more potent anticholinesterase diazoxon. The extent of toxicity mediated by this phenomenon is dependent on how fast diazoxon is hydrolyzed to less toxic metabolites, a process that is also accelerated by the enzyme induction. Similarly, concurrent exposure to diazinon and MFO enzyme-inhibiting substances (e.g., carbon monoxide; ethylisocyanide; SKF 525A, halogenated alkanes, such as $CC1_4$; alkenes, such as vinyl chloride; and allelic and acetylenic derivatives) may increase the toxicity of diazinon by decreasing the rate of the hydrolytic dealkylation and hydrolysis of both parent diazinon and activated diazinon (diazoxon) (Williams and Burson 1985). The balance between activation and detoxification determines the biological significance of these chemical interactions with diazinon.

Diazinon exposure may interfere with the short-acting muscle relaxant, succinylcholine, used concurrently with anesthetics. The action of succinylcholine is terminated by means of its hydrolysis by serum cholinesterase (Klaassen et al. 1986). Since serum cholinesterase is strongly inhibited by diazinon (Davies and Holub 1980b; Edson and Noakes 1960; Klemmer et al. 1978; Williams et al. 1959), it is possible that concurrent exposure to diazinon may result in the prolongation of the action of succinylcholine leading to prolonged muscular paralysis.

2.8 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to diazinon than will most persons exposed to the same level of diazinon in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the pre-existing compromised function of target organs (including effects or clearance rates and any resulting end-product metabolites). For these reasons we expect the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, Populations With Potentially High Exposure.

The magnitude of diazinon toxicity, like the toxicity of any xenobiotic, is affected by the rate of its metabolic biotransformation to both more and less toxic substances (Klaassen et al. 1986). Therefore, low xenobiotic metabolizing activity could result in greater toxicity. The newborn of several animal species, including humans, have a reduced ability to metabolize xenobiotics and may be more sensitive to diazinon toxicity.

Studies on experimental animals showed that starvation depressed liver microsomal enzyme (P-450) activity due to actual loss of the enzyme protein (Boyd and Carsky 1969). Thus, it is expected that dietary deficiency in protein would increase diazinon toxicity by diminishing its metabolism in the liver. Hereditary factors may also contribute to population sensitivity to diazinon. Atypical serum cholinesterase with low activity is present in a small percentage of the human population. This altered enzyme is the result of an hereditary factor with 0.04% occurrence in the population. Since serum cholinesterase is strongly inhibited by diazinon (Davies and Holub 1980b; Edson and Noakes 1960; Klemmer et al. 1978; Williams et al. 1959), it is expected that individuals who have atypical ChE (or low plasma cholinesterase activity) will be unusually sensitive to the muscle relaxant succinylcholine (Klaassen et al. 1986) and may suffer prolonged muscle paralysis if administered succinylcholine while exposed to diazinon. Congenital low plasma cholinesterase activity may also increase subpopulation sensitivity to diazinon exposure. This is because, after exposure, serum cholinesterase acts as a depot for diazinon due to its strong affinity for the substance (Davies and Holub 1980b; Edson and Noakes

1960; Klemmer et al. 1978; Williams et al. 1959), thus decreasing the availability of the diazinon dose to the target (neuromuscular tissue) of diazinon toxicity in the population with normal plasma cholinesterase levels. In individuals with congenital low plasma cholinesterase activity, less diazinon is bound in the blood and more unbound diazinon is in circulation to reach the target of diazinon toxicity (neuromuscular tissue).

2.9 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to diazinon. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to diazinon. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.9.1 Reducing Peak Absorption Following Exposure

Organophosphate insecticides like diazinon are rapidly absorbed after inhalation, ingestion, and dermal contact. In oral exposures, emesis is not indicated because of the danger of aspiration of stomach contents by an obtunded patient. Gastric lavage, with a solution of 5% sodium bicarbonate or 2% potassium permanganate, may be indicated within the first 60 minutes after ingestion to get rid of unabsorbed diazinon in the stomach (Shankar 1967, 1978). Activated charcoal can also be used but cathartics are not necessary due to the diarrhea induced by muscarinic activity. Decontamination is the first step in reducing dermal or eye contact absorption. This decontamination should begin immediately after the exposure is recognized. Contaminated clothing should be removed and skin (including hair and nails) should be washed copiously with soap and water. Health care workers and emergency responders should be protected from secondary contamination, and clothes and other contaminated material should be treated as contaminated waste. Eyes should be irrigated with copious amounts of room temperature water or saline, if available, for at least 15 minutes. irritation, lacrimation, or especially pain, swelling, and photophobia persist after 15 minutes of irrigation, expert ophthalmologic care should be sought.

If exposure is via inhalation, the exposed individual should be moved to fresh air and efforts should be directed toward the maintenance of an open airway, airway suctioning, endotracheal intubation. Artificial ventilation with supplemental oxygen may be helpful.

2.9.2 Reducing Body Burden

Diazinon is rapidly metabolized, with an estimated mammalian biological half-life of 12-15 hours (Iverson et al. 1975; Miicke et al. 1970). Consequently, efforts at reducing body burdens of poisoned persons may not be critical to the outcome. Dialysis and hemoperfusion are not indicated in organophosphate poisonings because of the extensive tissue distribution of the absorbed doses (Mucke et al. 1970; Poklis et al. 1980).

2.9.3 Interfering with the Mechanism of Action for Toxic Effects

As an anticholinesterase organophosphate, the principal toxic effect of diazinon in humans and laboratory animals derive from inhibition of neural acetylcholinesterase (Coye et al. 1987; Davies and Holub 1980a, 1980b; Edson and Noakes 1960; Enan et al. 1982; Harris et al. 1969; Rajendra et al. 1986; Takahashi et al. 1991; Wecker et al. 1985). Severe inhibition of this enzyme results in accumulation of acetylcholine at its sites of action and excessive or interminable stimulation of both sympathetic and parasympathetic cholinergic receptors leading to muscarinic and nicotinic effects, as manifested by muscular fasciculations, weakness, and paralysis; mydriasis; tachycardia; hypertension; miosis; excessive glandular secretions (salivation, lacrimation, rhinitis); nausea; urinary incontinence; vomiting; abdominal pain; diarrhea; bronchoconstriction or bronchospasm; increased bronchosecretion; vasodilation; bradycardia; hypotension; respiratory depression; anxiety; insomnia; headache; restlessness; tension; mental confusion; loss of concentration; apathy; drowsiness; ataxia; tremor; convulsion; and coma (Adlakha et al. 1988; Bichile et al. 1983; Coye et al. 1987; Kabrawala et al. 1965; Klaassen et al. 1986; Klemmer et al. 1978; Maizlish et al. 1987; Rayner et al. 1972; Shankar 1967, 1978; Williams and Burson 1985).

Timely treatment of diazinon poisoning cases with atropine and 2-PAM significantly reduces the cholinergic effects (Harris et al. 1969; Klemmer et al. 1978; Shankar 1967, 1978).

Atropine is an anti-muscarinic agent which, in large doses, alleviates bronchoconstriction and reduces secretion in the oral cavity and the airway. Atropine also counters some of the central nervous system

effects of organophosphates. Atropine should be given immediately by intravenous injection until evidence of "atropinization" or muscarinic blockade, such as flushing, dry mouth, dilated pupils, and tachycardia is seen. The most clinically important indication for continued atropine treatment is persistent wheezing (pulmonary rales) or bronchorrhea (Woo 1990). Pralidoxime (2-PAM) acts to regenerate inhibited acetylcholinesterase enzyme at all affected sites by displacing the diethylphosphoester bond diazinon forms at the active site. It should also be given immediately after diazinon poisoning is diagnosed and can be repeated to counter the nicotinic manifestations such as muscular weakness and fasciculations. Pralidoxime is most effective if started within the first 24 hours, preferably within 6-8 hours of exposure, prior to the irreversible phosphorylation of the enzyme (Schenker et al. 1992; Shankar 1967, 1978).

2.10 ADEQUACY OF THE DATABASE

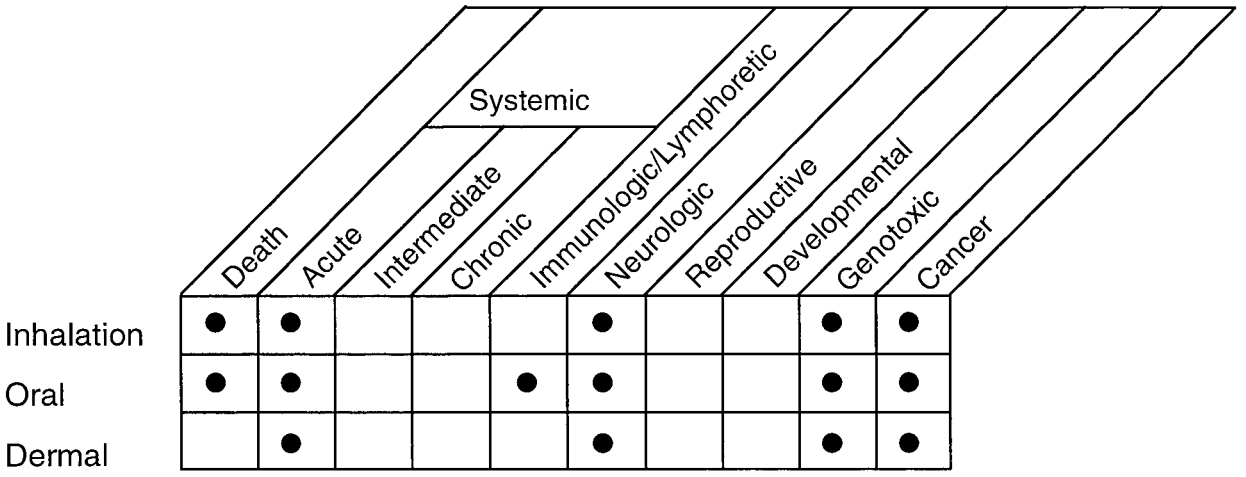
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of diazinon is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of diazinon.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

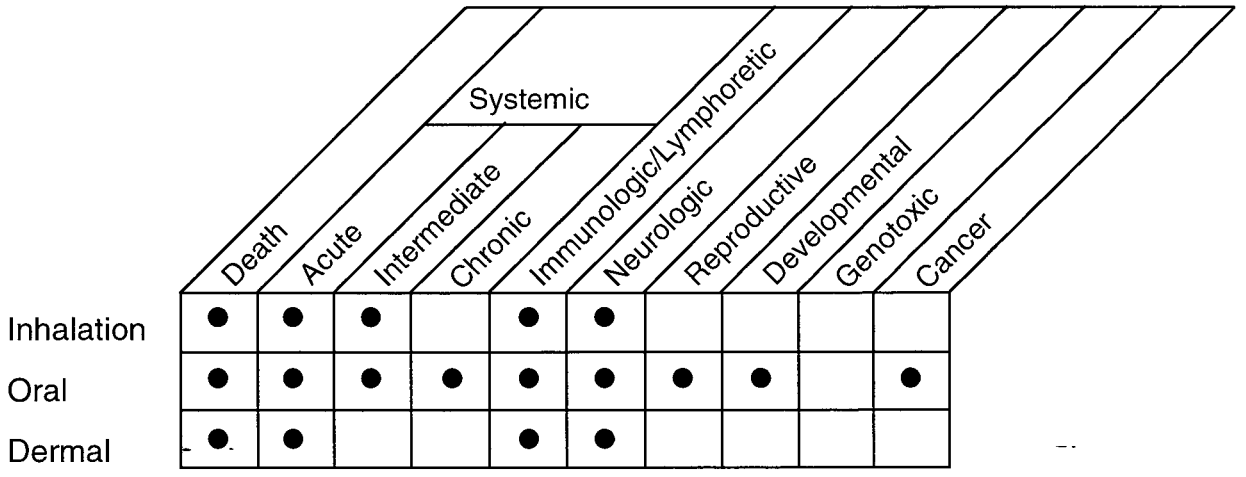
2.10.1 Existing Information on Health Effects of Diazinon

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to diazinon are summarized in Figure 2-4. The purpose of this figure is to illustrate the existing information concerning the health effects of diazinon. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure

Figure 2-4. Existing Information of Health Effects of Diazinon



Human



Animal

● Existing Studies

be interpreted as a "data need." A data need, as defined in *ATSDR's Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Most of the literature reviewed concerning the health effects of diazinon in humans described case reports of individuals or groups of individuals exposed either occupationally or in the home following intentional poisoning attempts or otherwise accidental misuse of diazinon or diazinon-containing solutions. The predominant route of occupational exposure is believed to be dermal while that for accidental or intentional exposure in the home is oral, although some inhalation exposures were reported. Thus, Figure 2-4 reflects that information exists for all three routes of exposure. However, all of these reports are limited because of the possibility of concurrent or sequential exposure to other potentially toxic substances present in the environment (workplace or home), such as other insecticides, or present as components of diazinon-containing formulations. In all cases, accurate information regarding levels and duration of exposure were not presented in these reports. Further, the health effects of human acute exposure to diazinon are much more fully characterized than those associated with intermediate and chronic exposures.

Information regarding the health effects of diazinon following ingestion in laboratory animals is substantial, but less information is available on the effects of inhalation and dermal exposures (see Figure 2-4). Furthermore, the health effects of acute- and intermediate-duration exposures to diazinon are more fully characterized than those associated with chronic-duration exposures. The available information indicates that diazinon is a toxic substance to all species of experimental animals, deriving its toxicity from acetylcholinesterase inhibition.

2.10.2 Identification of Data Needs

Acute-Duration Exposure. Information is available on the effects of acute-duration exposures in humans and experimental animals (rats and mice). The information available on humans consists primarily of studies of cholinergic (neurological) reactions resulting from acetylcholinesterase inhibition. Effects noted include respiratory, cardiovascular, hematological, kidney, liver, gastrointestinal tract, endocrine, neurological, and immunologic/lymphoreticular system toxicity (Balani

et al. 1968; Bichile et al. 1983; Dagli et al. 1981; De Palma et al. 1970; Hata et al. 1986; Kabrawala et al. 1965; Klemmer et al. 1978; Lee 1989; Limaye 1966; Lisi et al. 1987; Matsushita and Aoyama 1981; Poklis et al. 1980; Shankar 1967; Wadia et al. 1974; Wecker et al. 1985; Wedin et al. 1984; Weizman and Sofer 1992). The type of information available in animals includes LDsO values (Boyd and Carsky 1969; Edson and Noakes 1960; Enan et al. 1982; Gaines 1960, 1969; Harris et al. 1969; Krijnen and Boyd 1971; Noakes and Sanderson 1969) and cholinergic (neurological) reactions resulting from acetylcholinesterase inhibition. Effects noted include respiratory, gastrointestinal, hematological, liver, kidney, immunologic/lymphoreticular, and neurological toxicity (Boyd and Carsky 1969; Edson and Noakes 1960; Enan et al. 1982; Lox 1983; Mihara et al. 1981). Thus, while the acute effects of diazinon inhalation and oral exposure in humans are well-characterized and stem principally from acetylcholinesterase inhibition, the diazinon exposure levels at which these effects begin to occur are usually not known. Similarly, the available animal studies provide adequate insight into the acetylcholinesterase inhibiting action of diazinon in acute oral exposures, but lack sufficient dose-response data for estimating protective levels for humans. In addition, most of the earlier reports on diazinon did not report information on the purity of the test substance. This is especially important for diazinon where degradation of improperly stabilized diazinon has been associated with human toxicity (Hayes 1982). Quantitative acute oral exposure information as well as information on the acute inhalation toxicity and toxicokinetics in both humans and laboratory animals would be helpful in developing acute oral and inhalation MRLs for the protection of populations, especially those surrounding hazardous waste sites or establishments where wastes containing diazinon are released into the air or water, and those that are occupationally exposed to high levels of diazinon for brief periods.

Intermediate-Duration Exposure. Information is available on the effects of intermediate duration exposures in humans and experimental animals (rats, dogs, pigs). The type of information available includes studies of cardiovascular, gastrointestinal, hematological, musculoskeletal, renal, body weight, immunologic/lymphoreticular, and neurological effects (Anthony et al. 1986; Davies and Holub 1980a, 1980b; Earl et al. 1971; Enan et al. 1982; Lox and Davis 1983; Williams et al. 1959). Data from these-studies sufficiently demonstrate that diazinon is an anticholinesterase insecticide. The adverse effects reported in humans and laboratory animals following exposure via inhalation, dermal, or oral routes are predominately cholinergic responses deriving from erythrocyte or neuromuscular and central nervous system inhibition of acetylcholinesterase: MRLs of 0.009 mg/m^3 for inhalation exposure (Hartmann 1990) and 0.0002 mg/kg/day for oral exposure to diazinon (Barnes 1988) were derived from the database assembled for this profile. The MRLs are based on clear dose-response

effects of diazinon on the target for diazinon toxicity, neural acetylcholinesterase. Further information on the dermal toxicity and toxicokinetics for all routes in both humans and laboratory animals would be helpful for use in the assessment of intermediate-duration exposure protective levels, especially for persons near hazardous waste sites or establishments where wastes containing diazinon are released into the air, or near agricultural establishments where diazinon is used regularly.

Chronic-Duration Exposure and Cancer. No adequate epidemiological studies regarding the potential carcinogenicity or systemic toxicity of diazinon resulting from chronic exposure in humans are available. Two adequate studies have been conducted with rats and mice (NCI 1979). While not designed as a cancer bioassay, in a recent study where rats (groups of 20-30) were orally exposed to diazinon (Kirchner 1991) for 98 weeks, histopathology of some 30-40 different tissues showed no treatment-related increase in neoplasms. No chronic inhalation MRL was calculated for diazinon because no studies for this route are available. Toxicity and toxicokinetic data from well-conducted inhalation studies in both humans and laboratory animals would be helpful in developing a chronic inhalation MRL for the protection of populations, especially those surrounding hazardous waste sites or establishments where wastes containing diazinon are released into the air or water, and those that are occupationally exposed to diazinon for long periods of time.

Epidemiological studies available on diazinon are inadequate for assessing the carcinogenic potential of this chemical substance. The results from these studies are confounded by either concurrent or sequential (or both) exposures to other potentially toxic substances, mainly other insecticides (Cantor et al. 1992; Davis et al. 1993; Morris et al. 1986), although cancers in several tissue types (unspecified type of childhood brain cancer, non-Hodgkin's lymphoma, multiple myeloma) were identified in these chronic human exposure (presumed to be by several concurrent routes of exposure) studies. In adequate cancer oral bioassays conducted in rats and mice, the NCI (1979) concluded that diazinon is not carcinogenic in these species under the conditions of the bioassays. Chronic inhalation and dermal bioassays would be helpful to determine whether long-term inhalation or dermal exposures in populations, especially those surrounding hazardous waste sites or establishments where-wastes containing diazinon are released into the air or water, and those that are occupationally exposed to diazinon for long periods of time, are at risk of developing cancers.

Genotoxicity. Chronic occupational exposure to multiple insecticides, including diazinon, has been associated with an increased incidence of chromosomal aberration and increased sister chromatid

exchange in peripheral blood lymphocytes in these individuals (de Ferrari et al. 1991; Kiraly et al. 1979; See et al. 1990). The results from these studies are confounded by either concurrent or sequential (or both) exposures to other unknown toxic substances, mainly other insecticides, that may be genotoxic. No in viva genotoxicity studies in laboratory animals were located for diazinon. The results of *in vitro* tests in a variety of test systems (predominantly microbial assays) are equivocal. Diazinon was positive for gene mutations in one test using the *S. typhimurium* mutagenicity or reverse mutation assay with metabolic activation (Wong et al. 1989) and in the mouse lymphoma cell forward mutation assay without metabolic activation (McGregor et al. 1988). The compound was also positive for chromosomal aberrations in Chinese hamster cells with metabolic activation (Matsuoka et al. 1979). In contrast, evaluations for genetic mutation activity in the *S. typhimurium* mutagenicity or reverse mutation assay (Marshall et al. 1976) and in the ret-assay utilizing strains of B. subtilis (Shirasu et al. 1976) without metabolic activation, and in tests for sister chromatid exchange in Chinese hamster cells, both with and without metabolic activation (Chen et al. 1982), and for chromosomal aberrations in human peripheral blood lymphocytes (Lopez et al. 1986), were all negative. A full battery of *in vivo* tests in animals and additional *in vitro* tests in microbial systems for all genetic end points is necessary for the determination of the genetic toxicity potential of diazinon.

Reproductive Toxicity. No information was located on the reproductive effects of diazinon exposure in humans. Only three animal studies on the reproductive effects of diazinon exposure were located. The first study in rats actually found that oral diazinon exposure increased litter size (Green 1970), although a second rat study reported significant reduction ($p<0.05$) in litter size at oral maternal diazinon doses of 0.18 and 9 mg/kg/day (Spyker and Avery 1977). The third study, a 4-generation study which used only three dogs per sex, reported a dose-response testicular atrophy and arrested spermatogenesis in males in the fourth generation (Earl et al. 1971). Consequently, additional information on the reproductive effects in humans and animals is needed before the effect of diazinon exposure on human reproduction can be fully evaluated.

Developmental Toxicity. Information regarding the developmental effects in humans from exposure to diazinon was not located. Most of the located studies in laboratory animals did not find any significant developmental effects in the rats, mice, hamsters, and rabbits tested (Bamett et al. 1980; Green 1970; Robens 1969; Spyker and Avery 1977). In some of these studies, marked reduction in rat pup birth weight and continued significant retardation in growth rate (at 60 days,

F_4 treated rats weighed approximately only 50% as much as controls) (Green 1970), and significantly elevated ($p<0.05$) mortality in rat pups at weaning (Bamett et al. 1980) were reported. The effects reported for pups have been suggested to derive from diazinon impairment of placental transport of nutrients or maternal regulation of fetal growth, or directly via antagonism to cholinergic development of the fetus (Spyker and Avery 1977). Consequently, additional developmental studies in humans and animals by the inhalation and oral routes would be helpful in determining the human developmental toxicity of diazinon exposure.

Immunotoxicity. Autopsy reports in which the victims were exposed to high acute doses of diazinon described damage to immune structures (spleen, thymus) (Limaye 1966; Poklis et al. 1980). One study reported allergic interaction between the fungicide benomyl and diazinon from prolonged dermal contact with diazinon (Matsushita and Aoyama 198 1). Several oral animal studies also reported damage to immune structures in rats and dogs. Rats exhibited reduced spleen weight, splenic red pulp contraction, reduced thymus weight, and thymic atrophy ranging from minor to near total loss of thymocytes following acute exposure to moderate doses of diazinon (Boyd and Carsky 1969). A dose-response splenic degeneration after 232 days of diazinon exposure was also reported in one dog study in which only three dogs per sex were used (Earl et al. 1971). The splenic atrophy reported in this study may be a result of the generalized emaciated condition of the dog due to diarrhea, emesis, and anorexia. In other laboratory studies, exposure of guinea pigs in a dermal sensitization study, resulted in allergic interaction between the fungicide benomyl and diazinon (Matsushita and Aoyama 1981). Dermal application of diazinon induced delayed contact hypersensitivity at both 24 and 48 hours after challenge in the guinea pig maximization test (Matsushita et al. 1985). Evidently, diazinon has shown a potential to induce immunologic/lymphoreticular responses in laboratory animals. Additional human studies with diazinon would be helpful in defining the immunologic/ lymphoreticular injury potential of diazinon in humans.

Neurotoxicity. Available evidence shows that diazinon exposure in humans results in the inhibition of neural acetylcholinesterase (Coye et al. 1987; Davies and Holub 1980a, 1980b; Edson and Noakes 1960; Enan et al. 1982; Harris et al. 1969; Rajendra et al. 1986; Takahashi et al. 1991; Wecker et al. 1985). Severe inhibition of this enzyme results in accumulation of acetylcholine at its sites of action and excessive or interminable stimulation of both sympathetic and parasympathetic cholinergic receptors leading to muscarinic and nicotinic effects, as manifested by muscular fasciculations, weakness, and paralysis; mydriasis; tachycardia; hypertension; miosis; excessive glandular secretions

(salivation, lacrimation, rhinitis); nausea; urinary incontinence; vomiting; abdominal pain; diarrhea; bronchoconstriction or bronchospasm; increased bronchosecretion; vasodilation; bradycardia; hypotension; respiratory depression; anxiety; insomnia; headache; restlessness; tension; mental confusion; loss of concentration; apathy; drowsiness; ataxia; tremor; convulsion; and coma (Adlakha et al. 1988; Bichile et al. 1983; Coye et al. 1987; Kabrawala et al. 1965; Klaassen et al. 1986; Klemmer et al. 1978; Maizlish et al. 1987; Rayner et al. 1972; Shankar 1967, 1978; Williams and Burson 1985). These neurological effects have also been reported in laboratory animal studies in rats (Boyd and Carsky 1969; Earl et al. 1971). The current information from human and laboratory animal studies provides sufficient demonstration that the nervous system is the primary target of diazinon poisoning. However, the diazinon exposure levels at which these neurological effects begin to occur in chronic oral and inhalation exposures in humans and laboratory animals are not known. Therefore, additional studies designed to quantify acute and chronic LOAELs in humans and laboratory animals would be helpful for use in the development of MRLs for the protection of populations, especially those surrounding hazardous waste sites or establishments where wastes containing diazinon are released into the air or water, and those that are occupationally exposed to high levels of diazinon for brief periods or to lower concentrations for long periods.

Epidemiological and Human Dosimetry Studies. Available epidemiological studies sufficiently identify acetylcholinesterase inhibition as the characteristic and most critical effect from acute exposure to diazinon. However, these studies do not sufficiently identify the doses at which this effect occurs, although evidence from animal studies provides a measure of the lowest effect level (Coye et al. 1987; Davies and Holub 1980a, 1980b; Edson and Noakes 1960; Enan et al. 1982; Harris et al. 1969; Rajendra et al. 1986; Takahashi et al. 1991; Wecker et al. 1985). The results of an animal study indicated that more subtle effects of neuromuscular deficits in offspring of animals maternally exposed to doses lower than those that elicit acetylcholinesterase inhibition may be critical in assessing diazinon toxicity in humans. The authors of this animal study speculated that the mechanism of this reported effect is either indirect diazinon impairment of placental transport of nutrients or maternal regulation of fetal growth, or direct antagonism to cholinergic development of the fetus (Spyker and Avery 1977). The available epidemiological studies are inadequate for assessing the carcinogenic potential of this substance because the results from these studies are confounded by concurrent and/or sequential exposures to other potentially cancer-causing substances, mainly other insecticides (Cantor et al. 1992; Davis et al. 1993; Morris et al. 1986). These studies also lack adequate dose quantification. Additional chronic-duration oral, inhalation, and dermal studies would be helpful to

determine the acute-duration lowest-effect level for acetylcholinesterase inhibition and the effect on offspring of low-dose maternal exposure to diazinon during gestation, and to provide unequivocal data about the carcinogenic potential of diazinon in humans. This information would be useful in assessing the human health consequences of short- and long-term oral, inhalation, or dermal exposure in specific populations, especially those living around hazardous waste sites or establishments where wastes containing diazinon are released into the air or water, and those that are occupationally exposed to diazinon for long periods of time.

Biomarkers of Exposure and Effect.

Exposure. Diazinon is rapidly adsorbed from the gastrointestinal tract and widely distributed throughout the body in both humans (Poklis et al. 1980) and animals (Janes et al. 1973; Mticke et al. 1970). No human or animal studies have reported the presence of unchanged diazinon in the urine following exposure, although traces of unchanged diazinon have been detected in animal feces following exposure (Mticke et al. 1970). Diazinon undergoes biotransformation to a variety of polar metabolites which have been detected in the urine and feces of animals. Urinary and fecal excretion of 2-isopropyl-4-methyl-6-hydroxypyrimidine diethylphosphorothioic acid, and diethylphosphoric acid have been reported following oral exposure of animals to diazinon (Aizawa 1989; Iverson et al. 1975; Machin et al. 1975; Mount 1984; Mticke et al. 1970; Yang et al. 1971) while only diethylphosphorothioic acid and diethylphosphoric acid have been detected in the urine of exposed insecticide applicators (Maizlish et al. 1987). Although analysis of urine samples for the presence of these metabolites represents a potential means of assessing recent human exposure to diazinon, these metabolites can originate from exposure to other organophosphorus compounds and, therefore, are not specific for diazinon exposure. Additionally, these studies do not report a quantitative association between metabolite levels and exposure to diazinon in humans. Thus, these biomarkers are only indicative of exposure to diazinon (or other organophosphorus compounds) and are not specifically useful for diazinon exposure nor for dosimetric analysis. Further studies designed to refine the identification of-metabolites specific to diazinon and provide dosimetric data will be useful in the search for a more dependable biomarker of diazinon exposure.

Effect. The major action resulting from human exposure to diazinon is the inhibition of acetylcholinesterase (Coye et al. 1987; Davies and Holub 1980a, 1980b; Edson and Noakes 1960; Enan et al. 1982; Harris et al. 1969; Rajendra et al. 1986; Takahashi et al. 1991; Wecker et al. 1985). Two

pools of cholinesterases are present in human blood: acetylcholinesterase in erythrocytes and serum cholinesterase in plasma. Acetylcholinesterase, present in human erythrocytes, is identical to the enzyme present in neuromuscular tissue (the target of diazinon action). Inhibition of both forms of cholinesterase have been associated with exposure to diazinon in humans and animals (Coye et al. 1987; Edson and Noakes 1960; Soliman et al. 1982). While serum cholinesterase has no known physiological function, available data indicate that serum cholinesterase activity is a more sensitive marker for diazinon exposure than erythrocyte acetylcholinesterase activity (Endo et al. 1988; Hayes et al. 1980). Therefore, future studies that provide qualitative and dosimetric information regarding diazinon exposure and serum cholinesterase inhibition may provide a useful biomarker of effect for diazinon (or other anticholinesterase compounds) exposure. Currently, no effect specific to diazinon exposure has been identified by any study. Future studies designed to provide such information would be useful in identifying exposure to diazinon.

Absorption, Distribution, Metabolism, and Excretion. No studies were located regarding distribution and metabolism of diazinon after inhalation or dermal exposure in humans or animals, or regarding the excretion of diazinon after dermal exposure in animals. Diazinon was detected in several tissues from a woman who had ingested a lethal amount of a diazinon formulation, indicating rapid gastrointestinal tract absorption (Poklis et al. 1980). Animal studies also confirmed the rapid absorption of diazinon following oral administration (Abdelsalam and Ford 1986; Iverson et al. 1975; Janes et al. 1973; Machin et al. 1971, 1974; Mticke et al. 1970). Dermal absorption was estimated to be 34% of the applied dose with no difference related to the vehicle or to the area where it was applied in human volunteers dermally exposed for 24 hours to [^{14}C]diazinon applied to either the forearm or abdomen in acetone or lanolin wool grease (Wester et al. 1993). Absorbed diazinon was rapidly and widely distributed to body tissues in humans (Poklis et al. 1980). Animal studies confirmed the observation in humans (Mticke et al. 1970), in sheep (Abdelsalam and Ford 1986; Janes et al. 1973; Machin et al. 1971, 1974). Absorbed diazinon is metabolized extensively. Several metabolites (monoethyl phosphate, diethyl phosphate, diethyl phosphorothioate) and small amounts of unchanged diazinon -were found in the serum and urine of suicide victims who ingested diazinon (Klemmer et al. 1978; Poklis et al. 1980). Complete degradation of the pyrimidine ring does not take place; however, the ethyl side chain was completely degraded as indicated by results of a study conducted in rats. Detection of 2-isopropyl-4-methyl-6-hydroxypyrimidine and its oxidation products (oxidation at the primary and tertiary carbon atoms of the isopropyl side chains) indicates that the main degradative mechanism of diazinon is the hydrolysis of its ester bonds by cytochrome P-450

(Machin et al. 1975; Mucke et al. 1970). Diazinon is rapidly excreted in animals. Approximately 50-85% of diazinon doses were excreted, predominately via the urinary route, within 12-24 hours of dosing in animals. About 18-25% was excreted in the feces with a small amount expired in air (Iverson et al. 1975; Mount 1984; Miicke et al. 1970). Diazinon was not detected in the milk of lactating goats following oral administration (Mount 1984). Human volunteers excreted 34% of a dermally applied diazinon dose in the urine within 7 days (Wester et al. 1993). Additional studies in animals, designed to measure the rate of gastrointestinal absorption, distribution, and metabolism of diazinon after inhalation or dermal exposure in humans or animals, or regarding the excretion of diazinon after dermal exposure in animals would be useful in assessing the toxicokinetics of diazinon in humans, especially those living around hazardous waste sites or establishments where wastes containing diazinon are released into the air or water, and those that are occupationally exposed to diazinon for long periods of time.

Comparative Toxicokinetics. Diazinon, an anticholinesterase organophosphate, inhibits acetylcholinesterase in the central and peripheral nervous system resulting in cholinergic symptoms, in some cases. This effect has been reported in several human studies (Coye et al. 1987; Kabrawala et al. 1965; Klemmer et al. 1978; Limaye 1966; Maizlish et al. 1987; Rayner et al. 1972; Richter et al. 1992; Stalberg et al. 1978). Laboratory animal studies have confirmed this characteristic toxicity of diazinon. These animals also exhibited acetylcholinesterase inhibition and cholinergic response, in some cases (Boyd and Carsky 1969; Davies and Holub 1980a, 1980b; Earl et al. 1971; Edson and Noakes 1960; Harris et al. 1969; Takahashi et al. 1991). Although cholinergic symptoms and acetylcholinesterase inhibition resulted from human inhalation.and dermal exposures to diazinon (Coye et al. 1987; Lee 1989; Maizlish et al. 1987; Rayner et al. 1972; Richter et al. 1992; Soliman et al. 1982; Stalberg et al. 1978), no comparative animal studies are available. There is a correlation in the data regarding the absorption, distribution, metabolism, and excretion of diazinon following oral doses in both animal and human studies (Abdelsalam and Ford 1986; Iverson et al. 1975; Janes et al. 1973; Machin et al. 1971, 1974; Mount 1984; Miicke et al. 1970; Poklis et al. 1980); however, comparative data on the distribution and metabolism of diazinon after inhalation or dermal exposure in humans or animals, or on the excretion of diazinon after dermal exposure in animals are not available. Further studies are required to fill these data gaps.

Methods for Reducing Toxic Effects. Organophosphate insecticides like diazinon are rapidly absorbed and metabolized after inhalation, ingestion, and dermal contact (Iverson et al. 1975; Miicke et

al. 1970). Consequently, efforts at reducing body burdens of poisoned persons may not be critical to the outcome. In oral exposures, emesis is not indicated because of the danger of aspiration of stomach contents by an obtunded patient. Methods suggested for removing gastrointestinal tract diazinon content include gastric lavage and administration of activated charcoal (Shankar 1967, 1978). Cathartics are considered unnecessary. Dialysis and hemoperfusion are not indicated in organophosphate poisonings because of the extensive tissue distribution of the absorbed doses (Miicke et al. 1970; Poklis et al. 1980). In dermal or ocular exposures, thorough decontamination including discarding of contaminated clothing, copious washing of affected body parts with soap and water, and irrigation of the eyes with saline are recommended. Moving of victims to fresh air, airway suctioning, endotracheal intubation, and artificial ventilation with supplemental oxygen are recommended for inhalation exposures. Timely treatment of diazinon poisoning cases with atropine and 2-PAM significantly reduces the cholinergic effects (Harris et al. 1969; Klemmer et al. 1978; Shankar 1967, 1978). Atropine is an anti-muscarinic agent which, in large doses, alleviates bronchoconstriction and reduces secretion in the oral cavity and the airway induced by diazinon poisoning. Atropine also counters some of the central nervous system effects (Woo 1990). 2-PAM is given to regenerate inhibited cholinesterase (acetylcholinesterase) enzyme at all affected sites (Schenker et al. 1992; Shankar 1967, 1978). The available information sufficiently satisfies the need for methods of reducing toxic effects. Therefore, further studies in this regard are not required.

2.10.3 Ongoing Studies

The combined toxicity of commodation (the active ingredient in the anti-ulcer drug Tagamet) and the insecticide diazinon is being investigated in a study at the National Institute of Environmental Health Sciences in an effort to determine if simultaneous exposure to these two compounds might enhance the toxicity of diazinon. Results of these studies indicate that diazinon is very rapidly metabolized and that simultaneous exposure to commodation has little effect on the half-life of diazinon but does alter the ratio of some metabolites formed.

The Occupational Studies Section of the National Cancer Institute, Division of Cancer Etiology, is currently conducting epidemiologic studies to identify and quantify occupational causes of cancer. During the past year, investigations have uncovered associations between employment as a farmer and lymphatic and hematopoietic, skin, lip, brain, stomach, and prostate cancer. Use of several insecticides

including diazinon was linked with non-Hodgkin's lymphoma. Occupational groups with complex exposures under study include farmers and insecticide applicators.

The U.S. Department of Agriculture is sponsoring several studies on diazinon. The University of Minnesota is developing and maintaining a network of information resources on the uses, benefits, and hazards of insecticides placed under